

NOVEL FABH ENZYME, COMPOSITIONS CAPABLE OF BINDING TO SAID  
ENZYME AND METHODS OF USE THEREOF

Technical Field of the Invention

The invention relates to the identification of a novel enzyme active site and  
5 methods enabling the design and selection of inhibitors of that active site.

Background of the Invention

The pathway for the biosynthesis of saturated fatty acids is very similar in prokaryotes and eukaryotes. However, the organization of the biosynthetic apparatus is very different. Vertebrates possess a type I fatty acid synthase (FAS) in which all of the  
10 enzymatic activities are encoded on one multifunctional polypeptide, the mature protein being a homodimer. The acyl carrier protein (ACP) is an integral part of the complex. In contrast, in most bacterial and plant FASs (type II) each of the reactions are catalyzed by distinct monofunctional enzymes and the ACP is a discrete protein. Mycobacteria are unique in that they possess both type I and II FASs. There therefore appears to be  
15 considerable potential for selective inhibition of the bacterial systems by broad-spectrum antibacterial agents (Rock, C. & Cronan, J. 1996. *Biochimica et Biophysica Acta* 1302, 1-16; Jackowski, S. 1992. In *Emerging Targets in Antibacterial and Antifungal Chemotherapy*. Ed. J. Sutcliffe & N. Georgopapadakou. Chapman & Hall, New York; Jackowski, S. et al. (1989). *J. Biol. Chem.* 264, 7624-7629.)

20 The first step in the biosynthetic cycle is the condensation of malonyl-ACP with acetyl-CoA by FabH. Prior to this, malonyl-ACP is synthesized from ACP and malonyl-CoA by FabD, malonyl CoA:ACP transacylase. In subsequent rounds malonyl-ACP is condensed with the growing-chain acyl-ACP (FabB and FabF, synthases I and II respectively). The second step in the elongation cycle is ketoester reduction by NADPH-dependent  $\beta$ -ketoacyl-ACP reductase (FabG). Subsequent dehydration by  $\beta$ -hydroxyacyl-ACP dehydrase (either FabA or FabZ) leads to trans-2-enoyl-ACP which is in turn converted to acyl-ACP by enoyl-ACP reductase (FabI). Further rounds of this cycle, adding two carbon atoms per cycle, eventually lead to palmitoyl-ACP whereupon the cycle is stopped largely due to feedback inhibition of FabH and I by palmitoyl-ACP (Heath, et al,  
25 (1996), *J.Biol.Chem.* 271, 1833-1836).

30 Cerulenin and thiolactomycin are potent and selective inhibitors of bacterial fatty acid biosynthesis. Extensive work with these inhibitors has proved that this biosynthetic pathway is essential for bacterial viability. No marketed antibiotics are targeted against fatty acid biosynthesis, therefore it is unlikely that novel antibiotics would be rendered

inactive by known antibiotic resistance mechanisms. There is an unmet need for developing new classes of antibiotic compounds, such as those that target FabH.

FabH enzymes are of interest as potential targets for antibacterial agents.

There is a need in the art for novel FabH enzyme active sites and catalytic sequences to enable identification and structure-based design of inhibitors, which are useful in the treatment or prophylaxis of diseases, particularly diseases caused by bacteria which may share catalytic domains with those of the invention.

#### Summary of the Invention

In one aspect, the present invention provides a novel FabH enzyme active site 10 crystalline form.

In another aspect, the present invention provides a novel FabH composition characterized by the catalytic residues Cys112, His244 and Asn274.

In still another aspect, the present invention provides a novel FabH composition characterized by the active site of 33 amino acid residues (including the catalytic residues).

15 In yet another aspect, the invention provides a method for identifying inhibitors of the compositions described above which methods involve the steps of: providing the coordinates of the structure of the invention to a computerized modeling system; identifying compounds which will bind to the structure; and screening the compounds identified for FabH inhibitory bioactivity.

20 In a further aspect, the present invention provides an inhibitor of the catalytic activity of any composition bearing the catalytic domain described above.

Another aspect of this invention includes machine readable media encoded with data representing the coordinates of the three-dimensional structure of the FabH crystal.

25 Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

#### Brief Description of the Drawings

Fig. 1 provides the atomic coordinates of the *E. coli* FabH dimer.

Fig. 2 provides the atomic coordinates of the *E. coli* FabH monomer in complex with acetyl-CoA.

30 Fig. 3 provides a projection of the ribbon diagram of the *E. coli* FabH dimer. The two monomers are drawn with a light or dark gray shading. The catalytic Cys112 is shown in dark ball-and-stick model.

Fig. 4 provides the ribbon diagram of the *E. coli* FabH monomer with the catalytic residue Cys112 is shown in dark ball-and-stick model. The N- and C-termini are labeled.

Fig. 5 provides the stereoview of the  $\alpha$ -carbon superposition between the structures of FabH and FabF. FabH is drawn in a thin black line and FabF in a thick gray line.

Fig. 6 provides the ribbon diagram of the *E. coli* FabH monomer with acetylated Cys112 and the CoA molecule in black ball-and-stick model. The orientation of the view is the same as that of Fig. 4.

Fig. 7 provides the superposition of the *E. coli* FabH catalytic residues in comparison to those of FabF. FabH is drawn in thick gray lines and FabF in thin black lines. FabH residues are label Cys112, His244 and Asn274, which corresponds to Cys163, His303 and His340, respectively.

10 **Detailed Description of the Invention**

The present invention provides a novel *E. coli* FabH crystalline structure, a novel FabH active site, and methods of use of the crystalline form and active site to identify FabH inhibitor compounds (peptide, peptidomimetic or synthetic compositions) characterized by the ability to competitively inhibit binding to the active site of a FabH enzyme. Also 15 provided herein is a novel FabH crystalline structure in complex with the substrate acetyl-CoA, and the identification of acetyl-CoA interacting residues in FabH.

I. **The Novel FabH Crystalline Three-Dimensional Structure**

The present invention provides a novel FabH crystalline structure based on the *E. coli* FabH. The amino acid sequences of the FabH are provided in Table 1 as SEQ ID NO:1.

**TABLE 1**

	Met	Tyr	Thr	Lys	Ile	Ile	Gly	Thr	Gly	Ser	Tyr	Leu	Pro	Glu	Gln
1					5					10				15	
25															
	Val	Arg	Thr	Asn	Ala	Asp	Leu	Glu	Lys	Met	Val	Asp	Thr	Ser	Asp
16						20				25			30		
	Glu	Trp	Ile	Val	Thr	Arg	Thr	Gly	Ile	Arg	Glu	Arg	His	Ile	Ala
31						35				40			45		
30	Ala	Pro	Asn	Glu	Thr	Val	Ser	Thr	Met	Gly	Phe	Glu	Ala	Ala	Thr
46						50			55			60			
	Arg	Ala	Ile	Glu	Met	Ala	Gly	Ile	Glu	Lys	Asp	Gln	Ile	Gly	Leu
61						65			70			75			
	Ile	Val	Val	Ala	Thr	Thr	Ser	Ala	Thr	His	Ala	Phe	Pro	Ser	Ala

	76	80	85	90
	Ala Cys Gln Ile Gln Ser Met Leu Gly Ile Lys Gly Cys Pro Ala			
	91	95	100	105
	Phe Asp Val Ala Ala Ala Cys Ala Gly Phe Thr Tyr Ala Leu Ser			
5	106	110	115	120
	Val Ala Asp Gln Tyr Val Lys Ser Gly Ala Val Lys Tyr Ala Leu			
	121	125	130	135
	Val Val Gly Ser Asp Val Leu Ala Arg Thr Cys Asp Pro Thr Asp			
	136	140	145	150
10	Arg Gly Thr Ile Ile Phe Gly Asp Gly Ala Gly Ala Ala Val			
	151	155	160	165
	Leu Ala Ala Ser Glu Glu Pro Gly Ile Ile Ser Thr His Leu His			
	166	170	175	180
	Ala Asp Gly Ser Tyr Gly Glu Leu Leu Thr Leu Pro Asn Ala Asp			
15	181	185	190	195
	Arg Val Asn Pro Glu Asn Ser Ile His Leu Thr Met Ala Gly Asn			
	196	200	205	210
	Glu Val Phe Lys Val Ala Val Thr Glu Leu Ala His Ile Val Asp			
	211	215	220	225
20	Glu Thr Leu Ala Ala Asn Asn Leu Asp Arg Ser Gln Leu Asp Trp			
	226	230	235	240
	Leu Val Pro His Gln Ala Asn Leu Arg Ile Ile Ser Ala Thr Ala			
	241	245	250	255
	Lys Lys Leu Gly Met Ser Met Asp Asn Val Val Val Thr Leu Asp			
25	256	260	265	270
	Arg His Gly Asn Thr Ser Ala Ala Ser Val Pro Cys Ala Leu Asp			
	271	275	280	285
	Glu Ala Val Arg Asp Gly Arg Ile Lys Pro Gly Gln Leu Val Leu			
	286	290	295	300
30	Leu Glu Ala Phe Gly Gly Phe Thr Trp Gly Ser Ala Leu Val Arg Phe			
	301	305	310	317

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As illustrated herein, the crystal structure is a tightly associated FabH dimer. Each monomer has two structural domains: the N-terminal domain (residues 1-170 of SEQ ID

NO:1) and the C-terminal domain (residues 171-317 of SEQ ID NO:1). The two domains are similar in their overall fold: each contains a 5-stranded  $\beta$ -sheet sandwiched between  $\alpha$ -helices and covered by other  $\beta$ -strands,  $\alpha$ -helices and loops. The structural similarity between the two halves of the protein indicates that FabH is probably evolved from two 5 genes of similar origin. The active site of FabH is at the center of the FabH monomer, formed at the junction of the N- and C-terminal domains. While the core architecture of the *E. coli* FabH bears some similarity to that of the FabF (Huang, et al, (1998), *EMBO J.* 17, 1183-1191), large differences exist in the atomic positions of the core  $\beta$ -strands, and the structures outside of the core  $\beta$ -strand are completely different. With amino acid sequence 10 identity between FabH and FabF being below 20%, the large differences are well expected. Therefore, the crystalline structure of *E. coli* FabH is novel.

As described above, the *E. coli* FabH is a dimer, each monomer contains an active site. The dimer formation is essential for the FabH activity because the active site of a monomer is comprised of at least Phe87 of the other monomer in the dimer. The present 15 invention provides both a crystalline monomer and dimer structure of *E. coli* FabH. Inhibitors that perturb or interact with this dimer interface are another target for the design and selection of anti-bacterial agents.

According to the present invention, the crystal structure of *E. coli* FabH has been 20 resolved at 2.0  $\text{\AA}$  (crystal form 1), and its selenomethionine mutant protein in complex with acetyl-CoA has been determined at 1.9  $\text{\AA}$  (crystal form 2). The structure was determined using the methods of MAD phasing and molecular replacement, and refined to R-factors of 18.9% and 27%, respectively.

Further refinement of the atomic coordinates will change the numbers in Figure 1-2 and Tables I - III, refinement of the crystal structure from another crystal form will result in 25 a new set of coordinates. However, distances and angles in Tables II will remain the same within experimental errors, and relative conformation of residues in the active site will remain the same within experimental error. For example, the two independently determined monomers in our crystal form 1 and the monomer in crystal form 2 do not have identical numerical coordinates, but the structures of these three monomers have very similar 30 structures, and the spatial relationship between amino acid residues are considered the same within experimental error. In fact, we would consider any structure that can be superimposed onto that of FabH with an rms error of less than 1.5  $\text{\AA}$  on  $\alpha$ -carbon atoms being a close structural homologue and the same rms error but over all protein atoms being an identical structure. Figure 1 provides the atomic coordinates of the *E. coli* FabH dimer,

which contains 634 amino acids. Figure 2 provides the atomic coordinates of the *E. coli* FabH monomer in complex with acetyl-CoA, which contains 317 amino acids. The FabH enzyme is characterized by an active site which preferably contains a binding site for the first substrate acetyl-CoA and the second substrate malonyl-ACP. The catalytic residues in

5 FabH are Cys112, His244 and Asn274, compared to Cys163, His303 and His340 in FabF. The difference in catalytic residues is not only limited to their amino acid identity (His340 to Asn274 change), but also their relative spatial arrangement. While FabH Cys112 and Asn274 can be well superimposed onto FabF Cys163 and His340, His244 of FabH occupies a very different position from that of His303 of FabF. This indicated the catalytic

10 mechanisms of the two enzymes are very different. The crystal structure described herein was solved in the presence and absence of acetyl-CoA. We identified that the catalytic Cys112 has been covalently acetylated, and the product CoA is still bound to the active site. The bound CoA enabled us to identify the active site cavity, which is long and narrow and shaped nicely to bind the  $\beta$ -mercaptoethylamine-patotheinate arm of CoA. The structure of

15 the acetyl-CoA complex also revealed all the key residues that are interacting with CoA and lining the active site, which is identified as a set of 33 amino acid residues listed in Table I. For example, the adenine part of CoA is sandwiched between the side chains of Arg151 and Trp32. Our structures are determined in the absence of malonyl-ACP. However, the same acetyl-CoA binding cavity should bind malonyl-ACP as well because their active site

20 binding regions are very similar and there is no apparent additional entrance to the active site. Moreover, while the FabH molecular surface in general negatively charged, a region just outside of the active site cavity is positively charge. This surface is mainly comprised of three  $\alpha$ -helices (30-37, 209-231 and 248-258) and contains a number of positively charged amino acids (Arg36, Arg40, Lys214, His222, Arg235 Arg249, Lys256, Lys257).

25 Since the acyl-carrier protein (ACP) is known to be very acidic or negatively charged, it is reasonable to assume this surface being the ACP binding surface.

Table I provides the the atomic coordinates of the apo *E. coli* FabH structure in the active site (in crystal form 1). Solvent molecules are omitted here for clarity, but can be found in Fig. 1. Residue 487 is Phe87 from the other monomer.

**TABLE I**

	ATOM	RESIDUE	X	Y	Z	Occ	B
5	1 N	THR 28	-24.151	18.846	61.990	1.00	36.45
	2 CA	THR 28	-23.735	19.054	60.610	1.00	36.69
	3 CB	THR 28	-22.196	19.086	60.565	1.00	32.66
	4 OG1	THR 28	-21.760	20.076	59.636	1.00	33.79
10	5 CG2	THR 28	-21.645	17.737	60.183	1.00	27.40
	6 C	THR 28	-24.238	17.990	59.627	1.00	38.85
	7 O	THR 28	-24.732	16.923	60.023	1.00	42.97
	8 N	TRP 32	-24.091	20.068	53.681	1.00	30.06
	9 CA	TRP 32	-23.725	21.413	54.092	1.00	28.93
15	10 CB	TRP 32	-24.277	21.708	55.486	1.00	29.27
	11 CG	TRP 32	-24.036	23.126	55.939	1.00	31.13
	12 CD2	TRP 32	-22.895	23.622	56.644	1.00	32.44
	13 CE2	TRP 32	-23.118	25.005	56.890	1.00	35.25
	14 CE3	TRP 32	-21.707	23.038	57.096	1.00	32.45
20	15 CD1	TRP 32	-24.880	24.197	55.779	1.00	33.86
	16 NE1	TRP 32	-24.333	25.331	56.351	1.00	35.49
	17 CZ2	TRP 32	-22.200	25.800	57.565	1.00	35.24
	18 CZ3	TRP 32	-20.793	23.832	57.765	1.00	34.43
	19 CH2	TRP 32	-21.046	25.197	57.994	1.00	36.72
25	20 C	TRP 32	-22.203	21.582	54.091	1.00	27.24
	21 O	TRP 32	-21.675	22.617	53.674	1.00	26.75
	22 N	ILE 33	-21.503	20.566	54.581	1.00	26.32
	23 CA	ILE 33	-20.042	20.617	54.642	1.00	25.89
	24 CB	ILE 33	-19.459	19.370	55.333	1.00	25.18
30	25 CG2	ILE 33	-17.925	19.444	55.366	1.00	26.64
	26 CG1	ILE 33	-20.024	19.253	56.744	1.00	18.01
	27 CD1	ILE 33	-19.621	18.008	57.421	1.00	19.10
	28 C	ILE 33	-19.432	20.755	53.258	1.00	24.76
	29 O	ILE 33	-18.630	21.650	53.022	1.00	23.20
35	30 N	ARG 36	-20.198	24.159	51.621	1.00	26.35
	31 CA	ARG 36	-19.545	25.296	52.237	1.00	27.73
	32 CB	ARG 36	-20.083	25.473	53.649	1.00	34.96
	33 CG	ARG 36	-19.562	26.715	54.326	1.00	47.48
	34 CD	ARG 36	-20.581	27.250	55.290	1.00	56.04
40	35 NE	ARG 36	-21.775	27.729	54.600	1.00	63.48
	36 CZ	ARG 36	-22.490	28.780	54.996	1.00	67.12
	37 NH1	ARG 36	-23.564	29.153	54.303	1.00	67.75
	38 NH2	ARG 36	-22.127	29.465	56.082	1.00	68.27

**TABLE I - Cont.**

	<u>ATOM</u>	<u>RESIDUE</u>	<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
5	39	C ARG	36	-18.014	25.292	52.233	1.00 23.26
	40	O ARG	36	-17.386	26.346	52.208	1.00 21.26
	41	N THR	37	-17.423	24.103	52.214	1.00 20.79
	42	CA THR	37	-15.973	23.969	52.258	1.00 19.72
	43	CB THR	37	-15.549	23.164	53.509	1.00 20.01
	44	OG1 THR	37	-16.014	21.812	53.384	1.00 17.59
10	45	CG2 THR	37	-16.157	23.752	54.765	1.00 18.21
	46	C THR	37	-15.363	23.272	51.047	1.00 20.77
	47	O THR	37	-14.234	23.571	50.657	1.00 20.98
	48	N CYS	112	-0.698	28.695	58.467	1.00 12.58
	49	CA CYS	112	-0.984	28.096	57.174	1.00 11.86
	50	CB CYS	112	-2.457	28.264	56.808	1.00 10.86
15	51	SG CYS	112	-3.580	27.460	57.935	1.00 22.06
	52	C CYS	112	-0.126	28.620	56.037	1.00 10.86
	53	O CYS	112	-0.003	27.939	55.025	1.00 13.89
	54	N LEU	142	-3.033	20.066	62.705	1.00 16.58
	55	CA LEU	142	-4.063	20.954	63.207	1.00 17.95
	56	CB LEU	142	-4.281	22.159	62.287	1.00 15.72
20	57	CG LEU	142	-3.100	23.125	62.126	1.00 18.13
	58	CD1 LEU	142	-3.628	24.499	61.738	1.00 14.84
	59	CD2 LEU	142	-2.246	23.204	63.415	1.00 12.26
	60	C LEU	142	-5.396	20.321	63.598	1.00 17.45
	61	O LEU	142	-6.111	20.883	64.417	1.00 17.68
	62	N ARG	151	-17.927	23.092	65.249	1.00 22.20
25	63	CA ARG	151	-18.230	22.887	63.841	1.00 25.49
	64	CB ARG	151	-19.699	23.217	63.534	1.00 24.14
	65	CG ARG	151	-20.051	22.998	62.052	1.00 33.87
	66	CD ARG	151	-21.530	23.158	61.748	1.00 37.44
	67	NE ARG	151	-21.991	24.545	61.780	1.00 41.79
	68	CZ ARG	151	-23.272	24.897	61.737	1.00 44.63
30	69	NH1 ARG	151	-23.612	26.173	61.771	1.00 46.51
	70	NH2 ARG	151	-24.219	23.970	61.666	1.00 47.88
	71	C ARG	151	-17.304	23.634	62.868	1.00 26.00
	72	O ARG	151	-16.686	23.018	61.992	1.00 26.64
	73	N GLY	152	-17.164	24.940	63.077	1.00 24.63
	74	CA GLY	152	-16.353	25.769	62.201	1.00 23.08
35	75	C GLY	152	-14.912	25.371	61.944	1.00 22.21
	76	O GLY	152	-14.366	25.679	60.880	1.00 21.32
	77	N ILE	155	-14.484	20.649	60.878	1.00 18.82
	78	CA ILE	155	-14.866	20.149	59.564	1.00 18.77
	79	CB ILE	155	-16.223	20.733	59.071	1.00 17.77
	80	CG2 ILE	155	-17.365	20.321	60.018	1.00 12.79

**TABLE II - Cont.**

	<u>ATOM</u>	<u>RESIDUE</u>	<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
5	81	CG1 ILE	155	-16.127	22.249	58.924	1.00 15.46
	82	CD1 ILE	155	-17.339	22.892	58.331	1.00 20.95
	83	C ILE	155	-13.823	20.489	58.531	1.00 18.45
	84	O ILE	155	-13.819	19.909	57.461	1.00 21.51
	85	N ILE	156	-12.958	21.450	58.819	1.00 18.70
	86	CA ILE	156	-11.985	21.825	57.812	1.00 19.10
10	87	CB ILE	156	-11.999	23.375	57.499	1.00 24.79
	88	CG2 ILE	156	-13.391	23.974	57.563	1.00 23.59
	89	CG1 ILE	156	-11.095	24.139	58.438	1.00 24.77
	90	CD1 ILE	156	-9.886	24.631	57.730	1.00 27.97
	91	C ILE	156	-10.544	21.338	57.935	1.00 18.32
	92	O ILE	156	-9.922	21.071	56.918	1.00 18.31
15	93	N PHE	157	-10.005	21.200	59.142	1.00 16.26
	94	CA PHE	157	-8.611	20.780	59.280	1.00 15.33
	95	CB PHE	157	-7.984	21.371	60.551	1.00 15.71
	96	CG PHE	157	-7.868	22.858	60.523	1.00 19.05
	97	CD1 PHE	157	-8.814	23.654	61.158	1.00 19.74
	98	CD2 PHE	157	-6.844	23.476	59.814	1.00 15.77
20	99	CE1 PHE	157	-8.737	25.057	61.076	1.00 21.28
	100	CE2 PHE	157	-6.761	24.855	59.727	1.00 11.63
	101	CZ PHE	157	-7.701	25.650	60.351	1.00 17.65
	102	C PHE	157	-8.278	19.286	59.190	1.00 16.07
	103	O PHE	157	-9.045	18.413	59.622	1.00 17.36
	104	N LEU	189	-7.786	34.391	64.172	1.00 19.01
25	105	CA LEU	189	-7.338	33.021	63.922	1.00 19.46
	106	CB LEU	189	-6.897	32.907	62.463	1.00 23.06
	107	CG LEU	189	-6.422	31.587	61.872	1.00 23.21
	108	CD1 LEU	189	-7.435	30.493	62.157	1.00 24.24
	109	CD2 LEU	189	-6.253	31.811	60.355	1.00 25.52
	110	C LEU	189	-6.164	32.746	64.850	1.00 18.26
30	111	O LEU	189	-5.082	33.338	64.688	1.00 15.62
	112	N LEU	205	-7.765	25.549	68.834	1.00 19.58
	113	CA LEU	205	-7.699	26.448	67.677	1.00 19.69
	114	CB LEU	205	-7.475	25.601	66.398	1.00 19.66
	115	CG LEU	205	-7.104	26.238	65.052	1.00 19.36
	116	CD1 LEU	205	-6.309	25.259	64.201	1.00 18.01
35	117	CD2 LEU	205	-8.366	26.671	64.321	1.00 18.66
	118	C LEU	205	-8.996	27.273	67.597	1.00 17.98
	119	O LEU	205	-10.088	26.731	67.804	1.00 20.78
	120	N MET	207	-11.189	30.405	65.330	1.00 16.50
	121	CA MET	207	-11.285	31.040	64.025	1.00 18.56
	122	CB MET	207	-11.105	30.003	62.931	1.00 20.76

**TABLE II - Cont.**

	<u>ATOM</u>	<u>RESIDUE</u>		<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
5	123	CG	MET	207	-11.293	30.550	61.542	1.00 23.66
	124	SD	MET	207	-10.858	29.292	60.353	1.00 32.43
	125	CE	MET	207	-12.262	28.166	60.555	1.00 31.26
	126	C	MET	207	-12.599	31.742	63.776	1.00 18.83
	127	O	MET	207	-13.666	31.152	63.934	1.00 19.82
10	128	N	GLY	209	-14.190	32.425	61.134	1.00 20.42
	129	CA	GLY	209	-14.305	32.056	59.737	1.00 23.52
	130	C	GLY	209	-14.623	33.114	58.701	1.00 24.44
	131	O	GLY	209	-13.771	33.456	57.884	1.00 26.52
	132	N	ASN	210	-15.839	33.640	58.738	1.00 23.37
15	133	CA	ASN	210	-16.291	34.615	57.758	1.00 25.94
	134	CB	ASN	210	-17.724	35.006	58.035	1.00 24.49
	135	CG	ASN	210	-18.633	33.818	58.029	1.00 25.13
	136	OD1	ASN	210	-18.680	33.068	57.061	1.00 25.86
	137	ND2	ASN	210	-19.325	33.603	59.130	1.00 25.81
20	138	C	ASN	210	-15.426	35.831	57.639	1.00 26.62
	139	O	ASN	210	-15.214	36.334	56.545	1.00 27.59
	140	N	VAL	212	-12.110	35.950	58.414	1.00 25.15
	141	CA	VAL	212	-10.808	35.645	57.793	1.00 25.13
	142	CB	VAL	212	-10.004	34.469	58.486	1.00 23.52
25	143	CG1	VAL	212	-10.492	34.190	59.896	1.00 20.23
	144	CG2	VAL	212	-9.958	33.220	57.653	1.00 23.20
	145	C	VAL	212	-10.971	35.405	56.272	1.00 23.49
	146	O	VAL	212	-10.095	35.769	55.493	1.00 20.62
	147	N	PHE	213	-12.115	34.859	55.853	1.00 22.05
30	148	CA	PHE	213	-12.371	34.627	54.431	1.00 22.19
	149	CB	PHE	213	-13.718	33.954	54.244	1.00 20.95
	150	CG	PHE	213	-14.116	33.771	52.794	1.00 23.47
	151	CD1	PHE	213	-14.758	34.788	52.101	1.00 22.38
	152	CD2	PHE	213	-13.833	32.587	52.132	1.00 21.51
35	153	CE1	PHE	213	-15.098	34.634	50.784	1.00 23.71
	154	CE2	PHE	213	-14.173	32.423	50.813	1.00 26.06
	155	CZ	PHE	213	-14.805	33.446	50.133	1.00 25.34
	156	C	PHE	213	-12.307	35.935	53.645	1.00 22.07
	157	O	PHE	213	-11.618	36.045	52.629	1.00 22.83
40	158	N	ALA	216	-8.801	37.118	53.586	1.00 18.14
	159	CA	ALA	216	-7.964	36.216	52.808	1.00 19.00
	160	CB	ALA	216	-8.183	34.775	53.218	1.00 17.94
	161	C	ALA	216	-8.146	36.371	51.303	1.00 17.97
	162	O	ALA	216	-7.166	36.285	50.563	1.00 16.52
	163	N	LEU	220	-4.879	37.537	49.135	1.00 15.55
	164	CA	LEU	220	-4.379	36.571	48.174	1.00 17.76

**TABLE I - Cont.**

	<u>ATOM</u>	<u>RESIDUE</u>	<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
5	165	CB LEU	220	-5.127	35.233	48.275	1.00 15.75
	166	CG LEU	220	-4.703	34.362	49.466	1.00 13.65
	167	CD1 LEU	220	-5.621	33.177	49.608	1.00 13.89
	168	CD2 LEU	220	-3.278	33.915	49.310	1.00 9.45
	169	C LEU	220	-4.491	37.186	46.769	1.00 17.28
	170	O LEU	220	-3.618	36.957	45.932	1.00 20.62
10	171	N HIS	244	-3.012	27.197	48.689	1.00 18.90
	172	CA HIS	244	-3.165	26.587	49.988	1.00 17.18
	173	CB HIS	244	-2.914	27.594	51.111	1.00 17.15
	174	CG HIS	244	-3.178	27.035	52.465	1.00 17.42
	175	CD2 HIS	244	-2.579	26.015	53.138	1.00 14.15
	176	ND1 HIS	244	-4.285	27.385	53.212	1.00 16.36
15	177	CE1 HIS	244	-4.370	26.596	54.264	1.00 16.12
	178	NE2 HIS	244	-3.354	25.760	54.244	1.00 19.14
	179	C HIS	244	-4.631	26.151	49.971	1.00 15.41
	180	O HIS	244	-5.503	26.936	49.591	1.00 14.10
	181	N ALA	246	-7.440	26.051	51.721	1.00 19.76
	182	CA ALA	246	-8.240	26.512	52.864	1.00 20.02
20	183	CB ALA	246	-8.166	28.017	52.975	1.00 22.28
	184	C ALA	246	-9.687	26.075	52.772	1.00 23.08
	185	O ALA	246	-10.281	25.620	53.759	1.00 23.37
	186	N ASN	247	-10.280	26.349	51.615	1.00 21.20
	187	CA ASN	247	-11.645	25.983	51.311	1.00 22.48
	188	CB ASN	247	-12.653	26.733	52.190	1.00 24.84
25	189	CG ASN	247	-12.700	28.195	51.888	1.00 26.54
	190	OD1 ASN	247	-13.343	28.618	50.942	1.00 32.63
	191	ND2 ASN	247	-12.016	28.987	52.686	1.00 31.62
	192	C ASN	247	-11.824	26.292	49.825	1.00 23.43
	193	O ASN	247	-11.076	27.097	49.249	1.00 23.61
	194	N ARG	249	-14.126	27.939	48.119	1.00 27.79
30	195	CA ARG	249	-14.566	29.305	47.811	1.00 28.98
	196	CB ARG	249	-15.376	29.912	48.966	1.00 34.43
	197	CG ARG	249	-16.577	29.118	49.433	1.00 45.16
	198	CD ARG	249	-17.307	29.859	50.557	1.00 52.72
	199	NE ARG	249	-18.235	30.862	50.037	1.00 60.09
	200	CZ ARG	249	-18.607	31.976	50.675	1.00 62.73
35	201	NH1 ARG	249	-19.469	32.803	50.096	1.00 64.82
	202	NH2 ARG	249	-18.112	32.290	51.867	1.00 60.24
	203	C ARG	249	-13.369	30.208	47.562	1.00 24.80
	204	O ARG	249	-13.358	31.007	46.629	1.00 24.09
	205	N ILE	250	-12.393	30.135	48.453	1.00 24.48
	206	CA ILE	250	-11.201	30.951	48.306	1.00 24.93

**TABLE I - Cont.**

	<u>ATOM</u>	<u>RESIDUE</u>		<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
5	207	CB	ILE	250	-10.365	30.965	49.621	1.00 26.91
	208	CG2	ILE	250	-8.880	31.128	49.350	1.00 22.69
	209	CG1	ILE	250	-10.902	32.091	50.506	1.00 32.57
	210	CD1	ILE	250	-10.216	32.245	51.828	1.00 38.42
	211	C	ILE	250	-10.391	30.533	47.076	1.00 23.12
10	212	O	ILE	250	-10.024	31.380	46.265	1.00 20.24
	213	N	ASN	274	-7.884	20.993	55.104	1.00 16.04
	214	CA	ASN	274	-6.887	22.042	55.213	1.00 16.17
	215	CB	ASN	274	-7.524	23.307	55.790	1.00 16.71
	216	CG	ASN	274	-6.524	24.433	56.031	1.00 15.26
15	217	OD1	ASN	274	-5.290	24.259	55.970	1.00 14.69
	218	ND2	ASN	274	-7.058	25.607	56.319	1.00 17.12
	219	C	ASN	274	-5.800	21.538	56.144	1.00 18.93
	220	O	ASN	274	-6.016	21.456	57.366	1.00 18.02
	221	N	SER	276	-2.883	23.010	56.745	1.00 14.34
20	222	CA	SER	276	-1.996	24.086	57.152	1.00 14.51
	223	CB	SER	276	-1.772	23.993	58.686	1.00 16.80
	224	OG	SER	276	-1.051	25.104	59.218	1.00 17.07
	225	C	SER	276	-0.675	24.141	56.352	1.00 13.90
	226	O	SER	276	-0.719	24.199	55.132	1.00 15.64
25	227	N	ALA	303	-0.360	31.072	49.683	1.00 15.48
	228	CA	ALA	303	-0.934	30.617	50.937	1.00 13.31
	229	CB	ALA	303	0.045	29.692	51.624	1.00 11.10
	230	C	ALA	303	-1.261	31.801	51.853	1.00 12.59
	231	O	ALA	303	-0.614	32.842	51.789	1.00 11.22
30	232	N	PHE	304	-2.299	31.642	52.666	1.00 14.82
	233	CA	PHE	304	-2.726	32.650	53.626	1.00 15.34
	234	CB	PHE	304	-4.075	33.248	53.207	1.00 17.57
	235	CG	PHE	304	-4.561	34.355	54.119	1.00 23.99
	236	CD1	PHE	304	-5.356	34.060	55.243	1.00 22.77
35	237	CD2	PHE	304	-4.220	35.687	53.866	1.00 22.34
	238	CE1	PHE	304	-5.794	35.064	56.089	1.00 19.22
	239	CE2	PHE	304	-4.657	36.705	54.712	1.00 24.60
	240	CZ	PHE	304	-5.447	36.389	55.826	1.00 19.92
	241	C	PHE	304	-2.831	31.946	54.982	1.00 15.89
40	242	O	PHE	304	-3.176	30.768	55.041	1.00 14.69
	243	N	GLY	305	-2.490	32.637	56.065	1.00 14.20
	244	CA	GLY	305	-2.583	32.002	57.363	1.00 13.74
	245	C	GLY	305	-2.765	32.889	58.578	1.00 13.32
	246	O	GLY	305	-2.788	34.115	58.496	1.00 12.57
	247	N	GLY	306	-2.856	32.235	59.727	1.00 17.80

**TABLE I - Cont.**

	<u>ATOM</u>	<u>RESIDUE</u>		<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
5	248	CA	GLY	306	-3.033	32.929	60.990	1.00 17.87
	249	C	GLY	306	-1.928	33.892	61.357	1.00 19.45
	250	O	GLY	306	-0.758	33.751	60.965	1.00 19.00
	251	N	PHE	487	0.118	30.521	66.721	1.00 16.05
	252	CA	PHE	487	-0.254	31.597	65.800	1.00 15.49
	253	CB	PHE	487	-0.539	31.168	64.330	1.00 10.60
10	254	CG	PHE	487	-1.559	30.100	64.167	1.00 9.77
	255	CD1	PHE	487	-1.169	28.788	63.944	1.00 11.44
	256	CD2	PHE	487	-2.916	30.410	64.157	1.00 12.27
	257	CE1	PHE	487	-2.109	27.796	63.715	1.00 10.46
	258	CE2	PHE	487	-3.878	29.416	63.925	1.00 11.90
	259	CZ	PHE	487	-3.477	28.111	63.705	1.00 9.96
15	260	C	PHE	487	-1.381	32.376	66.460	1.00 13.45
	261	O	PHE	487	-2.233	31.776	67.132	1.00 15.58

Table II provides the distances between (D) atoms of the active site residues that are within 5.0 angstroms of one another as defined by Table I.

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**TABLE II**

	Distance						
	<u>Atom 1</u>	<u>Atom 2</u>	<u>Between (D=)</u>				
25	28CA	151CD	4.796	45	32N	33CA	4.198
	28CB	33CD1	4.204		32N	33C	4.728
	28CB	151CD	4.292		32N	33CB	4.967
	28CB	33CG1	4.398		32CA	33N	2.428
	28CB	151CG	4.703		32CA	33CA	3.808
	28OG1	33CG1	3.472		32CA	33C	4.422
30	28OG1	33CD1	3.709	50	32CA	33CB	4.890
	28OG1	151CD	3.743		32CB	33N	3.133
	28OG1	32CE3	3.902		32CB	33CA	4.454
	28OG1	151CG	4.159		32CG	33N	3.849
	28OG1	32CZ3	4.306		32CG	33CA	4.892
	28OG1	155CG2	4.418		32CD2	33N	3.941
35	28OG1	32CD2	4.776	55	32CD2	36CB	4.506
	28OG1	33CB	4.930		32CD2	36CD	4.511
	28OG1	151NE	4.962		32CD2	33CA	4.602
	28CG2	33CD1	3.435		32CD2	36NE	4.722
	28CG2	33CG1	4.093		32CE2	36CD	3.747
	32N	33N	2.785		32CE2	36NE	3.804

	32CE2	36CZ	4.270		32CZ3	33CG1	4.754
	32CE2	36CB	4.465		32CZ3	151CZ	4.802
	32CE2	36NH2	4.640	45	32CH2	36CD	3.427
	32CE2	36CG	4.706		32CH2	151NE	3.956
5	32CE2	151CZ	4.851		32CH2	36CG	4.238
	32CE2	36NH1	4.909		32CH2	36NE	4.297
	32CE3	33N	3.532		32CH2	151CD	4.299
	32CE3	33CA	3.828	50	32CH2	151CZ	4.365
	32CE3	33CG1	4.157		32CH2	155CD1	4.378
10	32CE3	36CB	4.522		32CH2	36CB	4.459
	32CE3	155CD1	4.542		32CH2	151NH1	4.669
	32CE3	33CB	4.649		32CH2	151CG	4.722
	32CE3	151CD	4.657	55	32CH2	36NH2	4.800
	32CE3	36CD	4.719		32CH2	36CZ	4.890
15	32CE3	151NE	4.929		32C	33CA	2.430
	32CD1	36NE	4.848		32C	33C	3.009
	32NE1	36NE	3.919		32C	33O	3.730
	32NE1	36CZ	4.139	60	32C	33CB	3.737
	32NE1	36CD	4.346		32C	36N	4.094
20	32NE1	36NH1	4.404		32C	33CG1	4.149
	32NE1	36NH2	4.693		32C	36CB	4.453
	32CZ2	36CD	3.146		32C	36CA	4.929
	32CZ2	36NE	3.563	65	32C	33CG2	4.950
	32CZ2	36CZ	3.945		32O	33N	2.249
25	32CZ2	36NH2	3.954		32O	33CA	2.757
	32CZ2	36CG	4.276		32O	33C	2.945
	32CZ2	151CZ	4.401		32O	36N	2.962
	32CZ2	151NE	4.403	70	32O	33O	3.261
	32CZ2	151NH1	4.452		32O	36CB	3.270
30	32CZ2	36CB	4.464		32O	36CA	3.712
	32CZ2	36NH1	4.873		32O	33CB	4.267
	32CZ2	151NH2	4.924		32O	36CG	4.657
	32CZ2	151CD	4.993	75	32O	37N	4.735
	32CZ3	155CD1	3.624		32O	36C	4.758
35	32CZ3	151CD	4.106		32O	33CG1	4.844
	32CZ3	36CD	4.225		33N	36N	4.835
	32CZ3	151NE	4.250		33CA	37OG1	4.386
	32CZ3	151CG	4.430	80	33CA	36N	4.658
	32CZ3	36CB	4.488		33CA	36CB	4.957
40	32CZ3	33CA	4.545		33CA	37N	4.991
	32CZ3	33N	4.616		33CA	37CG2	4.994
	32CZ3	36CG	4.653		33CB	37OG1	4.651

	33CG2	37OG1	3.631		36CB	37CG2	4.430
	33CG2	155CB	4.276		36CB	37CA	4.592
	33CG2	155CD1	4.585	45	36CG	37N	3.982
	33CG2	155O	4.633		36CG	37CG2	4.535
5	33CG2	37CG2	4.695		36CG	37CA	4.970
	33CG2	155CG2	4.767		36C	37CA	2.432
	33CG2	37CB	4.789		36C	37CG2	3.497
	33CG2	155CG1	4.874	50	36C	37CB	3.498
	33CG1	155CG2	4.351		36C	37C	3.538
10	33CG1	155CB	4.696		36C	37OG1	4.176
	33CG1	155CD1	4.793		36C	37O	4.442
	33CD1	155CG2	4.145		36C	249CD	4.916
	33CD1	155CB	4.658	55	36C	249CG	4.954
	33C	37OG1	3.580		36O	37N	2.243
15	33C	36N	3.854		36O	37CA	2.766
	33C	37N	4.042		36O	37CG2	3.844
	33C	37CB	4.576		36O	37C	3.859
	33C	36CA	4.656	60	36O	249CD	3.882
	33C	37CG2	4.688		36O	37CB	3.898
20	33C	36CB	4.779		36O	249CG	4.005
	33C	37CA	4.826		36O	37O	4.477
	33C	36C	4.863		36O	247CB	4.749
	33O	37OG1	2.646	65	36O	247OD1	4.807
	33O	37N	2.851		36O	37OG1	4.881
25	33O	36N	3.274		37CA	247CB	4.321
	33O	37CB	3.467		37CA	247CA	4.867
	33O	37CA	3.608		37CB	156CG2	4.663
	33O	37CG2	3.684	70	37CB	247CB	4.782
	33O	36C	3.777		37CG2	155CD1	3.854
30	33O	36CA	3.840		37CG2	156CG2	3.941
	33O	36CB	4.138		37CG2	155CG1	4.422
	33O	37C	4.148		37CG2	156CB	4.991
	33O	36O	4.926	75	37C	247CB	4.542
	36N	37N	2.838		37C	247CA	4.609
35	36N	37CA	4.277		37C	247C	4.810
	36N	37C	4.949		37O	247CA	3.598
	36CA	37N	2.434		37O	247C	3.729
	36CA	37CA	3.811	80	37O	247CB	3.853
	36CA	37CG2	4.500		37O	247N	4.926
40	36CA	37CB	4.704		37O	247O	4.938
	36CA	37C	4.796		112N	276OG	3.686
	36CB	37N	3.318		112N	305CA	3.963

112N	306N	4.333	112SG	304O	4.414		
112N	305C	4.677	112SG	157CE2	4.485		
112N	304O	4.709	45	112SG	274CG	4.632	
112N	276CB	4.828		112SG	276N	4.659	
5	112N	305N	4.952		112SG	305CA	4.685
	112N	276CA	4.966		112SG	276C	4.686
	112CA	276OG	3.624		112SG	244ND1	4.776
	112CA	276C	4.051	50	112SG	142CD1	4.820
	112CA	304O	4.061		112C	304O	3.861
10	112CA	276CA	4.136		112C	305CA	4.386
	112CA	305CA	4.225		112C	304C	4.415
	112CA	276O	4.408		112C	276C	4.524
	112CA	244NE2	4.434	55	112C	303CB	4.545
	112CA	276CB	4.443		112C	276O	4.551
15	112CA	244CE1	4.710		112C	244CD2	4.605
	112CA	304C	4.800		112C	305N	4.661
	112CA	244CD2	4.813		112C	244NE2	4.671
	112CA	305N	4.911	60	112C	276OG	4.831
	112CB	304O	3.148		112C	244CG	4.958
20	112CB	244CE1	3.594		112O	244CD2	3.728
	112CB	244NE2	3.694		112O	276O	3.809
	112CB	305CA	3.781		112O	303CB	3.827
	112CB	304C	4.127	65	112O	244NE2	4.073
	112CB	244ND1	4.129		112O	276C	4.079
25	112CB	276OG	4.216		112O	244CG	4.177
	112CB	276CA	4.217		112O	304O	4.251
	112CB	244CD2	4.306		112O	244CE1	4.632
	112CB	305N	4.436	70	112O	244ND1	4.683
	112CB	276C	4.515		112O	276CA	4.831
30	112CB	244CG	4.571		112O	244CB	4.890
	112CB	276CB	4.716		112O	304C	4.905
	112CB	276O	4.728		112O	304N	4.955
	112CB	306N	4.945	75	112O	303CA	4.975
	112CB	305C	4.962		142CA	157CB	4.754
35	112CB	274OD1	4.977		142CA	205CD1	4.956
	112SG	276OG	3.687		142CB	157CD2	3.797
	112SG	276CA	3.809		142CB	157CG	4.058
	112SG	244CE1	3.853	80	142CB	157CB	4.165
	112SG	276CB	3.982		142CB	205CD1	4.170
40	112SG	244NE2	4.070		142CB	157CE2	4.469
	112SG	274OD1	4.127		142CB	276CB	4.757
	112SG	274ND2	4.259		142CB	157CD1	4.905

	142CG	276CB	3.788		151C	155N	4.563
	142CG	276OG	4.071		151C	155CD1	4.597
	142CG	205CD1	4.377	45	151C	155CB	4.899
	142CG	157CD2	4.414		151O	152N	2.258
5	142CG	157CE2	4.706		151O	152CA	2.779
	142CD1	276CB	3.608		151O	152C	2.947
	142CD1	276OG	3.655		151O	155CG1	3.212
	142CD1	205CD1	3.719	50	151O	155CG2	3.411
	142CD1	157CE2	3.740		151O	155N	3.421
10	142CD1	157CD2	3.885		151O	152O	3.701
	142CD1	487CZ	4.116		151O	155CD1	3.721
	142CD1	487CE1	4.134		151O	155CB	3.737
	142CD1	157CZ	4.454	55	151O	155CA	4.176
	142CD1	157CG	4.706		152CA	155CG1	4.815
15	142CD1	276CA	4.885		152CA	155CD1	4.922
	142CD2	487CE1	4.604		152C	207CE	4.094
	142CD2	205CD1	4.620		152C	155CG1	4.510
	142CD2	276OG	4.759	60	152C	156CG2	4.843
	142CD2	276CB	4.818		152C	155N	4.860
20	142C	157CB	4.133		152O	207CE	3.274
	142C	157CG	4.691		152O	156CG2	3.855
	142O	157CB	4.323		152O	155CG1	4.323
	142O	205CD1	4.386	65	152O	156CG1	4.363
	142O	157CG	4.706		152O	156CB	4.727
25	151N	152N	2.952		152O	155CD1	4.807
	151N	152CA	4.351		152O	156N	4.911
	151CA	152N	2.436		155N	156N	2.685
	151CA	152CA	3.810	70	155N	156CA	4.127
	151CA	152C	4.558		155N	156CG2	4.821
30	151CA	155CG2	4.685		155N	157N	4.835
	151CB	152N	3.099		155N	156C	4.966
	151CB	152CA	4.414		155CA	156N	2.427
	151CG	152N	3.627	75	155CA	156CA	3.765
	151CG	155CG2	4.303		155CA	156CG2	4.562
35	151CG	155CD1	4.606		155CA	156C	4.769
	151CG	152CA	4.623		155CA	156CB	4.784
	151CD	152N	4.899		155CA	157N	4.991
	151C	152CA	2.431	80	155CB	156N	3.352
	151C	152C	3.097		155CB	156CA	4.554
40	151C	152O	4.095		155CB	156CG2	4.561
	151C	155CG1	4.343		155CG2	156N	4.705
	151C	155CG2	4.371		155CG1	156N	3.270

	155CG1 156CG2	3.509		156CG1 157CE2	4.578
	155CG1 156CA	4.310		156CG1 157CB	4.670
	155CG1 156CB	4.510	45	156CG1 207CE	4.697
	155CD1 156CG2	4.165		156CG1 274ND2	4.790
5	155CD1 156N	4.638		156CG1 246O	4.975
	155C 156CA	2.383		156CD1 274ND2	3.308
	155C 156C	3.439		156CD1 274CB	3.331
	155C 156CB	3.567	50	156CD1 157CZ	3.561
	155C 156CG2	3.643		156CD1 157CE1	3.563
10	155C 157N	3.931		156CD1 157N	3.712
	155C 156O	4.261		156CD1 157CE2	3.715
	155C 156CG1	4.558		156CD1 157CD1	3.722
	155O 156N	2.227	55	156CD1 274CG	3.772
	155O 156CA	2.675		156CD1 157CD2	3.864
15	155O 156C	3.604		156CD1 157CG	3.875
	155O 156CB	3.915		156CD1 246O	4.111
	155O 156CG2	4.089		156CD1 157CA	4.343
	155O 156O	4.103	60	156CD1 274CA	4.694
	155O 157N	4.363		156CD1 157CB	4.712
20	156N 157N	2.981		156CD1 274N	4.913
	156N 157CA	4.422		156CD1 274OD1	4.936
	156CA 157N	2.466		156C 157N	1.329
	156CA 157CA	3.825	65	156C 157CA	2.420
	156CA 157C	4.700		156C 157C	3.305
25	156CA 157O	4.854		156C 157CB	3.660
	156CA 157CB	4.870		156C 157O	3.694
	156CA 157CD1	4.959		156C 274N	3.900
	156CA 274N	4.980	70	156C 157CG	4.021
	156CB 157N	3.377		156C 274CB	4.195
30	156CB 157CA	4.624		156C 157CD1	4.329
	156CB 246O	4.688		156C 274O	4.565
	156CB 274CB	4.791		156C 274CA	4.613
	156CB 157CD1	4.859	75	156C 157CD2	4.668
	156CG2 157N	4.653		156O 157N	2.229
35	156CG1 157N	3.213		156O 157CA	2.717
	156CG1 157CD1	3.583		156O 274N	2.729
	156CG1 157CE1	3.655		156O 157C	3.324
	156CG1 157CG	4.050	80	156O 274CB	3.467
	156CG1 157CZ	4.179		156O 274CA	3.614
40	156CG1 157CA	4.262		156O 157O	3.892
	156CG1 157CD2	4.517		156O 274O	3.950
	156CG1 274CB	4.523		156O 157CB	4.129

1560	274C	4.220		157CZ	205CD1	4.113	
1560	157CG	4.518		157CZ	205CD2	4.153	
1560	274CG	4.862	45	157CZ	274CG	4.640	
1560	157CD2	4.863		157CZ	205CG	4.775	
5	157N	274O	4.374		157CZ	207SD	4.820
	157N	274N	4.566		157C	274O	3.627
	157N	274CB	4.672		157C	274N	4.446
	157CA	274O	3.295	50	157C	274C	4.527
	157CA	274N	4.244		1570	274O	4.850
10	157CA	274C	4.279		189N	207CA	4.847
	157CA	274CB	4.444		189CA	207CA	4.417
	157CA	274CA	4.594		189CA	207N	4.864
	157CB	274O	3.745	55	189CA	207CB	4.928
	157CB	274C	4.921		189CA	487CE2	4.997
15	157CG	274O	3.919		189CB	306CA	4.135
	157CG	205CD1	4.661		189CB	212CG1	4.600
	157CG	274CB	4.767		189CB	487CE2	4.841
	157CG	274CG	4.946	60	189CB	306N	4.926
	157CD1	205CD1	4.256		189CB	487CD2	4.995
20	157CD1	205CD2	4.394		189CG	306CA	3.750
	157CD1	205CG	4.976		189CG	487CE2	3.924
	157CD2	274O	3.280		189CG	306N	4.212
	157CD2	274CG	3.915	65	189CG	487CD2	4.347
	157CD2	274CB	4.085		189CG	487CZ	4.911
25	157CD2	274ND2	4.099		189CG	207CG	4.991
	157CD2	274OD1	4.220		189CD1	207CB	3.783
	157CD2	274C	4.280		189CD1	207CG	3.907
	157CD2	205CD1	4.766	70	189CD1	207SD	4.051
	157CD2	274CA	4.819		189CD1	487CE2	4.116
30	157CE1	205CD2	3.643		189CD1	207CA	4.314
	157CE1	205CD1	3.963		189CD1	205CD2	4.490
	157CE1	205CG	4.458		189CD1	487CZ	4.872
	157CE1	207CE	4.729	75	189CD1	207N	4.916
	157CE1	207SD	4.791		189CD1	487CD2	4.942
35	157CE2	274ND2	3.503		189CD2	306CA	3.467
	157CE2	274CG	3.728		189CD2	306N	3.480
	157CE2	274OD1	4.078		189CD2	305C	4.060
	157CE2	274O	4.205	80	189CD2	305O	4.557
	157CE2	274CB	4.299		189CD2	305CA	4.739
40	157CE2	205CD1	4.515		189CD2	212CG2	4.797
	157CE2	274C	4.976		189CD2	212CG1	4.883
	157CZ	274ND2	4.083		189CD2	306C	4.903

	189CD2	487CE2	4.911		209N	212N	4.914
	189CD2	212CB	4.963		209CA	210N	2.421
	189C	487CD2	4.060	45	209CA	210CA	3.796
	189C	487CE2	4.144		209CA	212CG1	4.372
5	189C	487O	4.648		209CA	210C	4.462
	189C	306CA	4.974		209CA	212N	4.662
	189O	487CD2	3.681		209CA	210CB	4.826
	189O	487O	4.066	50	209CA	212CG2	4.959
	189O	487CE2	4.173		209CA	210CG	4.975
10	189O	487C	4.215		209C	210CA	2.434
	189O	306CA	4.247		209C	210C	3.026
	189O	306C	4.621		209C	210CB	3.693
	189O	487CG	4.813	55	209C	212N	3.800
	205CG	487CZ	4.299		209C	210O	3.920
15	205CG	487CE2	4.667		209C	210CG	4.126
	205CD1	487CZ	4.050		209C	213N	4.177
	205CD1	487CE2	4.824		209C	210OD1	4.376
	205CD1	487CE1	4.931	60	209C	212CG1	4.433
	205CD2	207CB	4.532		209C	213CB	4.625
20	205CD2	207N	4.789		209C	212CA	4.667
	205C	207N	4.445		209C	210ND2	4.747
	205O	207N	4.564		209C	212CG2	4.782
	207CA	209N	4.326	65	209C	212CB	4.818
	207CB	209N	4.314		209C	212C	4.948
25	207CB	209CA	4.966		209O	210N	2.245
	207CG	209N	3.475		209O	210CA	2.777
	207CG	209CA	3.821		209O	210C	2.905
	207CG	212CG1	4.074	70	209O	213N	2.972
	207CG	212CG2	4.903		209O	212N	3.043
30	207SD	209CA	4.461		209O	210O	3.487
	207SD	209N	4.640		209O	213CB	3.674
	207SD	212CG2	4.851		209O	212CA	3.685
	207SD	212CG1	4.933	75	209O	212C	3.773
	207CE	209CA	4.469		209O	212CG2	3.827
35	207CE	209N	4.711		209O	213CA	3.906
	207C	209N	3.159		209O	212CG1	3.916
	207C	209CA	4.396		209O	212CB	3.947
	207O	209N	3.120	80	209O	210CB	4.249
	207O	209CA	4.341		209O	210CG	4.878
40	209N	210N	3.152		209O	212O	4.958
	209N	212CG1	4.281		209O	210OD1	4.993
	209N	210CA	4.540		210N	212N	4.398

210N	213N	4.866	212C	216CA	4.658		
210N	213CB	4.979	212C	213CG	4.966		
210CA	213CB	4.405	45	212O	213N	2.245	
210CA	212N	4.438		212O	216N	2.670	
5	210CA	213N	4.596		212O	213CA	2.759
	210C	213N	3.886		212O	213C	2.887
	210C	213CB	4.239		212O	216CB	3.134
	210C	213CA	4.591	50	212O	213O	3.255
	210C	212CA	4.624		212O	216CA	3.457
10	210C	212C	4.679		212O	213CB	4.240
	210O	213N	3.501		212O	304CE1	4.399
	210O	213CB	3.633		212O	216C	4.660
	210O	212N	3.644	55	212O	304CZ	4.701
	210O	213CA	3.933		213N	216N	4.607
15	210O	213C	4.126		213N	216CB	4.734
	210O	212C	4.352		213CA	250CD1	4.134
	210O	212CA	4.631		213CA	216CB	4.363
	210O	213CG	4.674	60	213CA	216N	4.434
	210O	213CD1	4.727		213CA	250CG1	4.898
20	212N	213N	2.784		213CA	216CA	4.958
	212N	213CA	4.205		213CB	250CD1	4.585
	212N	213C	4.773		213CG	250CG1	4.288
	212N	213CB	4.895	65	213CG	250CD1	4.298
	212CA	213N	2.468		213CG	249NH2	4.361
25	212CA	213CA	3.845		213CD1	249NH2	4.189
	212CA	213C	4.420		213CD1	250CG1	4.969
	212CA	216N	4.888		213CD1	249CZ	4.976
	212CA	213CB	4.891	70	213CD2	250CG1	3.388
	212CB	213N	3.397		213CD2	250CD1	3.646
30	212CB	213CA	4.698		213CD2	247ND2	4.070
	212CB	304CE1	4.881		213CD2	247OD1	4.172
	212CG1	213N	4.408		213CD2	249NH2	4.297
	212CG2	213N	3.253	75	213CD2	249CB	4.423
	212CG2	213CA	4.264		213CD2	247CG	4.542
35	212CG2	304CE1	4.815		213CD2	250CB	4.579
	212C	213N	1.335		213CD2	250N	4.650
	212C	213CA	2.440		213CD2	249CD	4.689
	212C	213C	2.994	80	213CD2	250CA	4.924
	212C	213CB	3.710		213CE1	249NH2	3.969
40	212C	213O	3.755		213CE1	249CZ	4.403
	212C	216N	3.855		213CE1	249NH1	4.789
	212C	216CB	4.183		213CE1	250CG1	4.914

213CE1	249NE	4.963		213O	213N	3.471	
213CE2	250CG1	3.302		213O	216CA	3.662	
213CE2	249CB	3.341	45	213O	216CB	3.709	
213CE2	250N	3.738		213O	216C	3.731	
5	213CE2	213CB	3.785		213O	250CD1	4.129
	213CE2	247OD1	3.897		213O	250CG1	4.545
	213CE2	249C	4.015		213O	216O	4.914
	213CE2	249CD	4.057	50	216N	304CZ	4.099
	213CE2	249NH2	4.080		216N	304CE2	4.314
10	213CE2	250CD1	4.089		216N	304CE1	4.419
	213CE2	250CA	4.157		216N	304CD2	4.807
	213CE2	250CB	4.248		216N	304CD1	4.895
	213CE2	249CG	4.314	55	216CA	304CE2	3.847
	213CE2	249CA	4.346		216CA	304CD2	3.926
15	213CE2	249NE	4.420		216CA	304CZ	3.934
	213CE2	249CZ	4.459		216CA	304CG	4.094
	213CE2	247ND2	4.468		216CA	304CE1	4.099
	213CE2	249O	4.492	60	216CA	304CD1	4.169
	213CE2	247CG	4.604		216CA	250CD1	4.669
20	213CZ	249CB	3.765		216CA	304CB	4.908
	213CZ	249NH2	3.909		216CA	220N	4.975
	213CZ	249CZ	4.112		216CA	220CD1	4.997
	213CZ	250CG1	4.148	65	216CB	250CD1	3.531
	213CZ	249NE	4.295		216CB	304CD1	3.550
25	213CZ	249C	4.377		216CB	304CE1	3.746
	213CZ	249CD	4.394		216CB	304CG	3.756
	213CZ	250N	4.428		216CB	304CZ	4.110
	213CZ	249O	4.508	70	216CB	304CD2	4.118
	213CZ	249NH1	4.708		216CB	304CE2	4.288
30	213CZ	249CG	4.729		216CB	304CB	4.383
	213CZ	250CA	4.749		216CB	250CG1	4.685
	213CZ	249CA	4.754		216CB	220CD1	4.706
	213C	216N	3.701	75	216C	220N	4.091
	213C	216CB	4.305		216C	220CG	4.389
35	213C	216CA	4.432		216C	220CD1	4.410
	213C	250CD1	4.614		216C	220CB	4.425
	213C	216C	4.795		216C	250CD1	4.646
	213O	216N	3.163	80	216C	304CD2	4.738
	213O	212O	3.255		216C	304CE2	4.889
40	213O	213CB	3.375		216C	220CA	4.901
	213O	213CG	3.382		216C	304CG	4.985
	213O	213CD1	3.423		216O	220N	2.973

216O	220CB	3.240		244CG	303CB	4.261	
216O	220CG	3.312		244CG	246N	4.437	
216O	220CD1	3.600	45	244CG	303CA	4.495	
216O	220CA	3.682		244CG	304O	4.536	
5	216O	304CD2	4.466		244CG	276O	4.605
	216O	220CD2	4.723		244CG	304N	4.694
	216O	220C	4.729		244CG	274OD1	4.945
	216O	304CG	4.812	50	244CD2	276O	3.276
	216O	304CE2	4.867		244CD2	276C	4.179
10	220CG	304CB	3.954		244CD2	274OD1	4.296
	220CG	304CD2	4.620		244CD2	276CA	4.491
	220CG	304CG	4.655		244CD2	276N	4.705
	220CG	304N	4.839	55	244CD2	303CB	4.764
	220CG	303C	4.910		244ND1	246N	3.736
15	220CG	304CA	4.914		244ND1	246CB	3.939
	220CG	303O	4.942		244ND1	304O	4.002
	220CD1	250CG2	3.858		244ND1	246CA	4.065
	220CD1	304CB	3.918	60	244ND1	274OD1	4.288
	220CD1	304N	4.769		244ND1	274ND2	4.528
20	220CD1	304CG	4.781		244ND1	274CG	4.656
	220CD1	304CA	4.980		244ND1	304N	4.729
	220CD2	303O	3.794		244CE1	274OD1	3.036
	220CD2	303C	3.874	65	244CE1	274ND2	3.525
	220CD2	304CB	4.033		244CE1	274CG	3.527
25	220CD2	303N	4.091		244CE1	246N	4.024
	220CD2	304N	4.170		244CE1	246CA	4.116
	220CD2	303CA	4.361		244CE1	246CB	4.253
	220CD2	304CA	4.531	70	244CE1	304O	4.409
	220CD2	304CD2	4.978		244CE1	276O	4.453
30	220CD2	304CG	4.997		244CE1	276CA	4.503
	244N	303CA	4.590		244CE1	276N	4.607
	244N	303N	4.800		244CE1	274CB	4.806
	244N	303CB	4.918	75	244CE1	276C	4.903
	244CA	246N	4.644		244NE2	274OD1	2.997
35	244CA	303CA	4.703		244NE2	276O	3.189
	244CA	303CB	4.756		244NE2	276CA	3.620
	244CB	303CA	3.618		244NE2	276N	3.747
	244CB	303CB	3.663	80	244NE2	276C	3.774
	244CB	304N	4.380		244NE2	274CG	3.873
40	244CB	303N	4.545		244NE2	274ND2	4.248
	244CB	303C	4.581		244NE2	246N	4.811
	244CB	246N	4.821		244C	246N	3.311

244C	246CA	4.639	246C	274CG	4.829
244O	246N	3.012	246O	247N	2.265
244O	246CA	4.288	45	246O	246CA
244O	246CB	4.440		246O	247CA
5	246N	247N	2.858	246O	247CB
	246N	247CA	4.225	246O	247ND2
	246N	247O	4.519	246O	247CG
	246N	274ND2	4.635	50	246O
	246N	274CG	4.694		274ND2
10	246N	247C	4.783		4.116
	246N	274CB	4.908	246O	247C
	246CA	247N	2.398	246O	274CG
	246CA	274ND2	3.762	246O	247O
	246CA	247CA	3.780	55	247CA
15	246CA	274CG	4.159	249N	4.491
	246CA	274CB	4.398	247CG	249N
	246CA	247CB	4.470	247CG	250CB
	246CA	247ND2	4.518	60	247CG
	246CA	247O	4.632	249CB	4.318
20	246CA	247C	4.704	247CG	250CG1
	246CA	274OD1	4.840	249CA	4.619
	246CA	247CG	4.866	247CG	249CG
	246CB	247N	3.017	247CG	250CD1
	246CB	247ND2	3.981	65	247CG
25	246CB	274ND2	4.268	250CA	4.762
	246CB	247CA	4.360	247CG	249C
	246CB	247CG	4.666	247OD1	249N
	246CB	247CB	4.733	250N	3.007
	246CB	247O	4.816	247OD1	250N
30	246CB	250CG2	4.830	247OD1	249CB
	246CB	250CD1	4.837	247OD1	249CA
	246CB	250CB	4.978	247OD1	250CB
	246CB	274CG	4.988	75	247OD1
	246C	247CA	2.445	249CD	3.431
35	246C	247CB	3.093	247OD1	250CG1
	246C	247C	3.647	247OD1	249O
	246C	247ND2	3.730	247ND2	250CD1
	246C	247CG	3.789	80	247ND2
	246C	247O	3.922	250CG1	3.870
40	246C	274ND2	4.440	247ND2	250CB
	246C	274CB	4.631	250N	4.930
	246C	247OD1	4.815	247ND2	250CA
				249N	4.004
				247C	4.402
				249N	4.869
				3.305	

247C	250N	4.120		274O	276CA	4.809	
247C	249CA	4.545		303N	304CA	4.862	
247C	249C	4.779	45	303CA	304N	2.430	
247C	250CB	4.900		303CA	304CA	3.818	
5	247C	250CA	4.940		303CA	304C	4.661
	247O	249N	3.360		303CA	304O	4.679
	247O	250N	3.406		303CA	304CB	4.684
	247O	250CB	3.950	50	303CB	304N	3.222
	247O	250CA	3.970		303CB	304CA	4.521
10	247O	250C	4.123		303CB	304O	4.818
	247O	249C	4.217		303CB	304C	4.963
	247O	249CA	4.373		303C	304CA	2.452
	247O	250CG2	4.591	55	303C	304CB	3.442
	249N	250N	2.817		303C	304C	3.504
15	249N	250CA	4.203		303C	304O	3.860
	249N	250C	4.666		303C	305N	4.467
	249CA	250N	2.413		303C	304CG	4.748
	249CA	250CA	3.779	60	303O	304N	2.247
	249CA	250C	4.413		303O	304CA	2.806
20	249CA	250CB	4.866		303O	304CB	3.762
	249CB	250N	3.035		303O	304C	3.989
	249CB	250CA	4.353		303O	304O	4.630
	249CG	250N	4.416	65	303O	305N	4.674
	249C	250CA	2.410		303O	304CG	4.827
25	249C	250C	3.035		304N	305N	3.547
	249C	250CB	3.720		304N	305CA	4.719
	249C	250O	3.774		304CA	305N	2.450
	249C	250CG1	4.278	70	304CA	305CA	3.795
	249C	250CG2	4.919		304CA	305C	4.958
30	249O	250N	2.240		304CB	305N	3.325
	249O	250CA	2.733		304CG	305N	3.321
	249O	250C	3.038		304CG	305CA	4.469
	249O	250O	3.374	75	304CG	305O	4.729
	249O	250CB	4.232		304CD1	305N	3.304
35	249O	250CG1	4.716		304CD1	305CA	4.052
	274OD1	276CB	4.452		304CD1	305O	4.145
	274OD1	276C	4.632		304CD1	305C	4.383
	274OD1	276O	4.648	80	304CD2	305N	4.139
	274ND2	276N	4.935		304CE1	305O	3.966
40	274C	276N	3.322		304CE1	305N	4.100
	274C	276CA	4.688		304CE1	305C	4.483
	274O	276N	3.552		304CE1	305CA	4.616

	304CE2	305N	4.804
	304CE2	305O	4.952
	304CZ	305O	4.401
	304CZ	305N	4.783
5	304C	305N	1.329
	304C	305CA	2.395
	304C	305C	3.718
	304C	305O	4.130
	304C	306N	4.754
10	304O	305N	2.239
	304O	305CA	2.696
	304O	305C	4.145
	304O	305O	4.826
	304O	306N	4.921
15	305N	306N	3.702
	305N	306CA	4.963
	305CA	306N	2.391
	305CA	306CA	3.771
	305CA	306O	4.400
20	305CA	306C	4.467
	305C	306CA	2.427
	305C	306C	3.071
	305C	306O	3.236
	305O	306N	2.248
25	305O	306CA	2.772
	305O	306C	2.996
	305O	306O	3.217
	306N	487CD2	4.792
	306CA	487CD2	4.048
30	306CA	487CG	4.502
	306CA	487CB	4.525
	306CA	487CE2	4.655
	306C	487CB	4.265
	306C	487CD2	4.576
35	306C	487CG	4.734
	306O	487CB	4.248
	306O	487CG	4.922

Table III provides the the atomic coordinates of the acetyl-CoA complex structure in the active site. Solvent molecules are omitted here for clarity, but can be found in Fig. 2. Residue 487 is Phe87 from the other monomer. Residue CAC is acetylated cysteine, and COA is the bound CoA molecule.

5

TABLE III

	<u>ATOM</u>	<u>RESIDUE</u>	<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
10	1	N THR	28	32.909	0.319	26.935	1.00 14.64
	2	CA THR	28	31.524	0.759	27.053	1.00 16.73
	3	CB THR	28	31.399	2.311	26.861	1.00 18.66
	4	OG1 THR	28	30.140	2.771	27.368	1.00 21.07
	5	CG2 THR	28	31.523	2.702	25.394	1.00 14.87
	6	C THR	28	30.671	-0.021	26.041	1.00 15.95
	7	O THR	28	31.196	-0.755	25.190	1.00 14.39
	8	N TRP	32	24.685	1.112	27.156	1.00 18.61
15	9	CA TRP	32	24.896	1.996	28.316	1.00 17.67
	10	CB TRP	32	26.253	1.657	28.999	1.00 18.46
	11	CG TRP	32	26.543	2.508	30.252	1.00 14.22
	12	CD2 TRP	32	26.947	3.865	30.325	1.00 16.45
	13	CE2 TRP	32	27.044	4.089	31.715	1.00 13.95
	14	CE3 TRP	32	27.232	4.916	29.444	1.00 14.91
	15	CD1 TRP	32	26.405	1.970	31.509	1.00 19.11
	16	NE1 TRP	32	26.722	2.948	32.369	1.00 17.55
20	17	CZ2 TRP	32	27.417	5.348	32.222	1.00 16.49
	18	CZ3 TRP	32	27.602	6.164	29.953	1.00 8.45
	19	CH2 TRP	32	27.698	6.373	31.321	1.00 11.56
	20	C TRP	32	24.917	3.414	27.781	1.00 16.08
	21	O TRP	32	24.363	4.325	28.378	1.00 17.69
	22	N ILE	33	25.536	3.534	26.593	1.00 16.72
	23	CA ILE	33	25.591	4.911	26.052	1.00 17.89
	24	CB ILE	33	26.670	5.169	24.944	1.00 20.24
25	25	CG2 ILE	33	26.790	6.671	24.704	1.00 18.87
	26	CG1 ILE	33	28.038	4.571	25.295	1.00 16.21
	27	CD1 ILE	33	28.930	4.480	24.013	1.00 24.09
	28	C ILE	33	24.196	5.403	25.732	1.00 18.98
	29	O ILE	33	23.877	6.540	26.194	1.00 18.61
	30	N ARG	36	22.046	6.096	28.836	1.00 20.61
	31	CA ARG	36	22.587	7.077	29.780	1.00 20.93
	32	CB ARG	36	23.940	6.602	30.339	1.00 19.27
35	33	CG ARG	36	23.882	5.328	31.146	1.00 20.40
	34	CD ARG	36	23.627	5.619	32.605	1.00 22.27
	35	NE ARG	36	23.511	4.396	33.393	1.00 27.02
	36	CZ ARG	36	23.867	4.298	34.670	1.00 25.93
	37	NH1 ARG	36	23.734	3.152	35.315	1.00 26.63

Table III Cont.

	<u>ATOM</u>	<u>RESIDUE</u>		<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
5	38	NH2 ARG	36	24.330	5.355	35.318	1.00	23.35
	39	C ARG	36	22.702	8.517	29.247	1.00	18.28
	40	O ARG	36	22.703	9.462	30.029	1.00	17.41
	41	N THR	37	22.798	8.697	27.936	1.00	18.97
	42	CA THR	37	22.932	10.050	27.405	1.00	21.02
10	43	CB THR	37	24.388	10.371	26.949	1.00	18.78
	44	OG1 THR	37	24.793	9.461	25.925	1.00	17.72
	45	CG2 THR	37	25.347	10.293	28.084	1.00	21.35
	46	C THR	37	22.048	10.362	26.222	1.00	20.16
	47	O THR	37	21.914	11.534	25.839	1.00	25.43
15	48	N CAC	112	30.456	25.709	28.104	1.00	10.38
	49	CA CAC	112	29.270	25.229	27.412	1.00	14.44
	50	CB CAC	112	28.799	23.888	27.980	1.00	17.69
	51	SG CAC	112	29.712	22.439	27.254	1.00	17.65
	52	CD CAC	112	32.183	21.508	28.594	1.00	24.17
20	53	CE CAC	112	30.937	22.403	28.616	1.00	21.28
	54	OE CAC	112	30.752	23.125	29.602	1.00	25.29
	55	C CAC	112	28.167	26.294	27.295	1.00	11.81
	56	O CAC	112	27.369	26.232	26.368	1.00	10.19
	57	N LEU	142	35.611	19.985	21.261	1.00	10.22
25	58	CA LEU	142	35.860	19.347	22.539	1.00	13.06
	59	CB LEU	142	34.735	19.597	23.555	1.00	12.36
	60	CG LEU	142	34.583	20.999	24.171	1.00	11.62
	61	CD1 LEU	142	33.937	20.919	25.543	1.00	5.06
	62	CD2 LEU	142	35.940	21.651	24.300	1.00	10.88
30	63	C LEU	142	36.175	17.851	22.433	1.00	13.55
	64	O LEU	142	36.786	17.299	23.322	1.00	19.07
	65	N ARG	151	36.295	6.724	29.164	1.00	23.03
	66	CA ARG	151	34.919	6.417	28.730	1.00	23.11
	67	CB ARG	151	34.470	5.004	29.175	1.00	16.86
35	68	CG ARG	151	34.348	4.774	30.666	1.00	15.32
	69	CD ARG	151	33.926	3.335	30.928	1.00	7.13
	70	NE ARG	151	33.779	3.086	32.349	1.00	10.71
	71	CZ ARG	151	33.378	1.927	32.869	1.00	3.91
	72	NH1 ARG	151	33.268	1.783	34.179	1.00	4.61
40	73	NH2 ARG	151	33.078	0.930	32.071	1.00	10.10
	74	C ARG	151	33.873	7.478	29.120	1.00	17.49
	75	O ARG	151	33.012	7.828	28.317	1.00	17.71
	76	N GLY	152	34.016	8.044	30.309	1.00	17.52
	77	CA GLY	152	33.070	9.045	30.776	1.00	16.37
	78	C GLY	152	33.062	10.401	30.082	1.00	15.84
	79	O GLY	152	32.246	11.248	30.439	1.00	21.56

Table III Cont.

	<u>ATOM</u>	<u>RESIDUE</u>	<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
5	80	N ILE	155	32.443	9.844	25.187	1.00 7.71
	81	CA ILE	155	31.083	9.426	24.707	1.00 12.55
	82	CB ILE	155	30.385	8.425	25.708	1.00 11.77
	83	CG2 ILE	155	31.197	7.148	25.866	1.00 11.90
	84	CG1 ILE	155	30.158	9.085	27.088	1.00 12.15
	85	CD1 ILE	155	29.158	8.276	27.966	1.00 11.79
10	86	C ILE	155	30.193	10.622	24.373	1.00 10.55
	87	O ILE	155	29.530	10.593	23.314	1.00 14.21
	88	N ILE	156	30.115	11.601	25.228	1.00 15.15
	89	CA ILE	156	29.284	12.781	24.971	1.00 13.87
	90	CB ILE	156	28.912	13.460	26.383	1.00 18.45
	91	CG2 ILE	156	27.632	12.860	26.931	1.00 23.09
15	92	CG1 ILE	156	30.082	13.252	27.370	1.00 15.34
	93	CD1 ILE	156	29.617	12.611	28.714	1.00 19.30
	94	C ILE	156	29.845	13.826	24.026	1.00 13.98
	95	O ILE	156	29.049	14.365	23.211	1.00 9.76
	96	N PHE	157	31.114	14.104	24.000	1.00 10.77
	97	CA PHE	157	31.656	15.152	23.157	1.00 7.33
20	98	CB PHE	157	32.859	15.790	23.759	1.00 4.54
	99	CG PHE	157	32.560	16.451	25.090	1.00 7.66
	100	CD1 PHE	157	32.946	15.788	26.255	1.00 3.98
	101	CD2 PHE	157	31.915	17.650	25.184	1.00 5.65
	102	CE1 PHE	157	32.660	16.349	27.491	1.00 9.88
	103	CE2 PHE	157	31.630	18.205	26.422	1.00 4.05
25	104	CZ PHE	157	32.018	17.536	27.588	1.00 6.80
	105	C PHE	157	31.810	14.851	21.690	1.00 10.70
	106	O PHE	157	32.380	13.859	21.257	1.00 13.03
	107	N LEU	189	34.231	20.663	36.441	1.00 15.69
	108	CA LEU	189	34.309	20.542	34.989	1.00 15.11
	109	CB LEU	189	32.983	20.982	34.350	1.00 10.07
30	110	CG LEU	189	32.807	20.922	32.844	1.00 7.51
	111	CD1 LEU	189	33.311	19.593	32.263	1.00 10.35
	112	CD2 LEU	189	31.343	21.142	32.523	1.00 7.61
	113	C LEU	189	35.464	21.418	34.538	1.00 15.40
	114	O LEU	189	35.452	22.612	34.812	1.00 16.51
	115	N LEU	205	40.306	17.390	29.143	1.00 13.16
35	116	CA LEU	205	39.050	17.802	29.770	1.00 15.27
	117	CB LEU	205	37.963	17.874	28.694	1.00 12.62
	118	CG LEU	205	36.505	18.215	29.034	1.00 14.99
	119	CD1 LEU	205	35.817	18.527	27.706	1.00 15.12
	120	CD2 LEU	205	35.773	17.085	29.762	1.00 11.51
	121	C LEU	205	38.658	16.793	30.846	1.00 15.81

**Table III Cont.**

	<u>ATOM</u>	<u>RESIDUE</u>	<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
5	122	O LEU	205	38.675	15.588	30.594	1.00 20.20
	123	N MET	207	35.792	15.888	34.121	1.00 18.42
	124	CA MET	207	34.419	16.232	34.463	1.00 16.18
	125	CB MET	207	33.555	16.227	33.174	1.00 17.87
	126	CG MET	207	32.024	16.237	33.467	1.00 17.17
	127	SD MET	207	30.990	16.464	32.044	2.00 17.60
10	128	CE MET	207	31.340	14.797	31.582	1.00 22.99
	129	C MET	207	33.790	15.238	35.466	1.00 16.62
	130	O MET	207	33.726	14.046	35.222	1.00 18.22
	131	N GLY	209	30.811	14.103	36.169	1.00 12.42
	132	CA GLY	209	29.492	14.040	35.588	1.00 16.72
	133	C GLY	209	28.358	14.011	36.516	1.00 19.06
15	134	O GLY	209	27.487	14.883	36.423	1.00 20.59
	135	N ASN	210	28.284	13.037	37.418	1.00 21.24
	136	CA ASN	210	27.150	13.010	38.362	1.00 24.44
	137	CB ASN	210	27.198	11.753	39.171	1.00 25.49
	138	CG ASN	210	27.160	11.958	40.631	1.00 33.50
	139	OD1 ASN	210	26.177	11.619	41.309	1.00 34.80
20	140	ND2 ASN	210	28.217	12.429	41.247	1.00 32.41
	141	C ASN	210	26.970	14.201	39.196	1.00 25.55
	142	O ASN	210	25.858	14.799	39.270	1.00 27.11
	143	N VAL	212	27.967	17.255	38.323	1.00 18.86
	144	CA VAL	212	27.657	18.365	37.397	1.00 19.45
	145	CB VAL	212	28.483	18.363	36.115	1.00 13.66
25	146	CG1 VAL	212	28.142	19.417	35.091	1.00 10.49
	147	CG2 VAL	212	29.921	18.642	36.480	1.00 11.31
	148	C VAL	212	26.176	18.359	36.977	1.00 25.20
	149	O VAL	212	25.455	19.359	36.929	1.00 27.15
	150	N PHE	213	25.738	17.114	36.763	1.00 24.63
	151	CA PHE	213	24.361	16.813	36.336	1.00 25.87
30	152	CB PHE	213	24.203	15.287	36.398	1.00 23.74
	153	CG PHE	213	22.788	14.958	36.099	1.00 23.97
	154	CD1 PHE	213	22.533	14.398	34.810	1.00 27.08
	155	CD2 PHE	213	21.752	14.909	36.974	1.00 22.61
	156	CE1 PHE	213	21.275	13.964	34.464	1.00 23.26
	157	CE2 PHE	213	20.480	14.482	36.625	1.00 23.27
35	158	CZ PHE	213	20.223	13.976	35.335	1.00 21.74
	159	C PHE	213	23.356	17.458	37.319	1.00 26.46
	160	O PHE	213	22.395	18.091	36.945	1.00 28.12
	161	N ALA	216	23.435	21.215	37.390	1.00 21.80
	162	CA ALA	216	22.949	21.675	36.100	1.00 19.74
	163	CB ALA	216	23.464	20.861	34.933	1.00 18.25

Table III Cont.

	<u>ATOM</u>	<u>RESIDUE</u>	<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
	164	C ALA	216	21.440	21.811	36.028	1.00 20.86
	165	O ALA	216	20.936	22.882	35.612	1.00 14.72
5	166	N HIS	244	21.005	23.509	25.764	1.00 14.90
	167	CA HIS	244	22.348	23.098	25.390	1.00 17.43
	168	CB HIS	244	23.328	23.551	26.478	1.00 17.97
	169	CG HIS	244	24.644	22.836	26.459	1.00 18.58
	170	CD2 HIS	244	25.582	22.714	25.488	1.00 18.43
10	171	ND1 HIS	244	25.123	22.136	27.546	1.00 18.75
	172	CE1 HIS	244	26.295	21.608	27.243	1.00 21.88
	173	NE2 HIS	244	26.597	21.944	26.000	1.00 17.34
	174	C HIS	244	22.190	21.563	25.366	1.00 17.94
	175	O HIS	244	21.579	20.979	26.286	1.00 18.08
15	176	N ALA	246	23.569	18.461	26.118	1.00 19.92
	177	CA ALA	246	24.594	17.753	26.886	1.00 22.75
	178	CB ALA	246	24.851	18.474	28.207	1.00 20.40
	179	C ALA	246	24.197	16.301	27.174	1.00 25.65
	180	O ALA	246	24.941	15.364	26.869	1.00 27.18
20	181	N ASN	247	23.035	16.122	27.793	1.00 26.14
	182	CA ASN	247	22.545	14.795	28.146	1.00 26.38
	183	CB ASN	247	22.964	14.464	29.587	1.00 28.11
	184	CG ASN	247	22.574	13.044	30.019	1.00 32.46
	185	OD1 ASN	247	21.552	12.486	29.583	1.00 30.09
25	186	ND2 ASN	247	23.371	12.470	30.912	1.00 31.19
	187	C ASN	247	21.021	14.827	28.020	1.00 26.38
	188	O ASN	247	20.381	15.783	28.497	1.00 24.78
	189	N ARG	249	18.806	13.418	29.619	1.00 26.17
	190	CA ARG	249	18.082	13.368	30.918	1.00 30.19
30	191	CB ARG	249	18.684	12.450	31.892	1.00 35.11
	192	CG ARG	249	20.149	12.514	32.084	1.00 40.00
	193	CD ARG	249	20.737	11.377	33.040	1.00 40.00
	194	NE ARG	249	19.770	10.270	32.981	1.00 40.00
	195	CZ ARG	249	20.131	8.991	32.720	1.00 40.00
35	196	NH1 ARG	249	19.206	8.005	32.728	1.00 40.00
	197	NH2 ARG	249	21.400	8.728	32.469	1.00 40.00
	198	C ARG	249	17.883	14.720	31.505	1.00 30.27
	199	O ARG	249	16.848	15.128	31.999	1.00 29.21
	200	N ILE	250	19.022	15.485	31.317	1.00 31.13
40	201	CA ILE	250	18.989	16.891	31.777	1.00 30.26
	202	CB ILE	250	20.417	17.557	31.646	1.00 31.49
	203	CG2 ILE	250	20.269	19.060	31.848	1.00 27.57
	204	CG1 ILE	250	21.391	16.967	32.703	1.00 27.31
	205	CD1 ILE	250	22.804	17.587	32.626	1.00 29.25

Table III Cont.

	<u>ATOM</u>	<u>RESIDUE</u>	<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
5	206	C ILE	250	17.878	17.652	31.051	1.00 29.32
	207	O ILE	250	17.014	18.274	31.667	1.00 30.29
	208	N ASN	274	27.325	16.399	22.555	1.00 13.47
	209	CA ASN	274	27.474	17.720	23.155	1.00 13.39
	210	CB ASN	274	27.818	17.530	24.622	1.00 15.56
10	211	CG ASN	274	27.960	18.816	25.366	1.00 17.87
	212	OD1 ASN	274	28.135	19.881	24.780	1.00 24.67
	213	ND2 ASN	274	27.890	18.729	26.689	1.00 19.64
	214	C ASN	274	28.638	18.414	22.458	1.00 14.33
	215	O ASN	274	29.770	17.971	22.613	1.00 12.94
15	216	N SER	276	29.549	21.633	22.863	1.00 7.51
	217	CA SER	276	29.823	22.861	23.613	1.00 13.37
	218	CB SER	276	31.354	23.045	23.758	1.00 16.08
	219	OG SER	276	31.709	24.178	24.552	1.00 13.44
	220	C SER	276	29.132	24.114	23.029	1.00 13.89
20	221	O SER	276	27.945	24.062	22.700	1.00 11.72
	222	N PHE	304	24.334	25.567	30.088	1.00 14.66
	223	CA PHE	304	25.107	25.471	31.332	1.00 17.36
	224	CB PHE	304	24.396	24.476	32.274	1.00 14.19
	225	CG PHE	304	25.035	24.321	33.630	1.00 14.80
25	226	CD1 PHE	304	26.179	23.562	33.795	1.00 13.55
	227	CD2 PHE	304	24.464	24.909	34.748	1.00 18.11
	228	CE1 PHE	304	26.751	23.388	35.053	1.00 13.17
	229	CE2 PHE	304	25.024	24.744	36.014	1.00 19.56
	230	CZ PHE	304	26.175	23.977	36.166	1.00 18.61
30	231	C PHE	304	26.495	24.936	30.934	1.00 18.48
	232	O PHE	304	26.597	24.072	30.048	1.00 19.82
	233	N GLY	305	27.546	25.411	31.603	1.00 20.16
	234	CA GLY	305	28.889	24.966	31.272	1.00 18.15
	235	C GLY	305	29.950	25.008	32.367	1.00 15.06
35	236	O GLY	305	29.701	25.407	33.507	1.00 11.78
	237	N GLY	306	31.145	24.556	31.988	1.00 16.84
	238	CA GLY	306	32.290	24.514	32.875	1.00 16.87
	239	C GLY	306	32.529	25.856	33.525	1.00 19.36
	240	O GLY	306	32.236	26.899	32.934	1.00 17.63
40	241	N PHE	487	38.425	26.469	30.807	1.00 13.64
	242	CA PHE	487	37.277	26.474	31.704	1.00 12.94
	243	CB PHE	487	35.953	26.064	31.031	1.00 16.12
	244	CG PHE	487	35.967	24.728	30.332	1.00 10.50
	245	CD1 PHE	487	36.055	24.668	28.952	1.00 11.96
	246	CD2 PHE	487	35.776	23.548	31.043	1.00 14.59
	247	CE1 PHE	487	35.943	23.450	28.275	1.00 11.67

Table III Cont.

	<u>ATOM</u>	<u>RESIDUE</u>	<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Occ</u>	<u>B</u>
	248	CE2 PHE	487	35.665	22.321	30.373	1.00 10.83
	249	CZ PHE	487	35.748	22.283	28.989	1.00 9.41
5	250	C PHE	487	37.606	25.574	32.861	1.00 13.75
	251	O PHE	487	38.217	24.529	32.661	1.00 18.53
	252	AO6 COA	350	25.886	9.541	33.559	1.00 40.00
	253	AP2 COA	350	25.938	8.466	34.779	1.00 40.00
	254	AO4 COA	350	25.984	7.033	34.193	1.00 40.00
10	255	AO5 COA	350	24.688	8.689	35.674	1.00 40.00
	256	AO3 COA	350	27.383	8.800	35.491	1.00 40.00
	257	AP1 COA	350	27.959	7.998	36.780	1.00 40.00
	258	AO1 COA	350	26.887	7.993	37.879	1.00 40.00
	259	AO2 COA	350	29.237	8.653	37.296	1.00 40.00
15	260	AO5* COA	350	28.201	6.460	36.164	1.00 40.00
	261	AC5* COA	350	27.718	5.279	36.817	1.00 39.18
	262	AC4* COA	350	28.472	4.019	36.378	1.00 37.65
	263	AO4* COA	350	28.702	4.012	34.931	1.00 35.45
	264	AC3* COA	350	29.898	3.856	36.965	1.00 37.54
20	265	AO3* COA	350	30.205	2.474	37.178	1.00 40.00
	266	AP3* COA	350	31.518	2.029	38.160	1.00 40.00
	267	AO7 COA	350	32.888	2.220	37.337	1.00 40.00
	268	AO8 COA	350	31.503	3.018	39.420	1.00 40.00
	269	AO9 COA	350	31.296	0.500	38.522	1.00 40.00
25	270	AC2* COA	350	30.688	4.469	35.850	1.00 32.65
	271	AO2* COA	350	32.112	4.433	35.932	1.00 24.96
	272	AC1* COA	350	30.098	3.815	34.584	1.00 27.72
	273	AN9 COA	350	30.429	4.564	33.382	1.00 20.99
	274	AC8 COA	350	30.840	5.878	33.186	1.00 21.31
30	275	AN7 COA	350	30.992	6.002	31.788	1.00 18.53
	276	AC5 COA	350	30.700	4.873	31.234	1.00 12.67
	277	AC6 COA	350	30.698	4.501	29.898	1.00 12.21
	278	AN6 COA	350	31.039	5.381	28.963	1.00 15.81
	279	AN1 COA	350	30.338	3.249	29.672	1.00 17.72
35	280	AC2 COA	350	30.014	2.442	30.654	1.00 11.38
	281	AN3 COA	350	29.997	2.743	31.973	1.00 15.08
	282	AC4 COA	350	30.341	3.964	32.268	1.00 15.56
	283	PS1 COA	350	27.926	20.647	30.314	1.00 40.00
	284	PC2 COA	350	26.439	19.897	31.045	1.00 40.00
40	285	PC3 COA	350	26.760	18.654	31.835	1.00 40.00
	286	PN4 COA	350	26.965	17.518	30.873	1.00 40.00
	287	PC5 COA	350	27.350	16.338	31.273	1.00 40.00
	288	PO5 COA	350	27.542	15.370	30.476	1.00 40.00
	289	PC6 COA	350	27.580	16.199	32.745	1.00 40.00

**Table III Cont.**

ATOM	RESIDUE		X	Y	Z	Occ	B	
290	PC7	COA	350	26.255	15.801	33.363	1.00 40.00	
291	PN8	COA	350	26.292	14.370	33.634	1.00 40.00	
5	292	PC9	COA	350	26.176	13.440	32.669	1.00 37.37
	293	PO9	COA	350	25.948	13.691	31.437	1.00 31.87
	294	PC10	COA	350	26.320	11.982	33.151	1.00 38.48
	295	PO10	COA	350	26.849	11.940	34.496	1.00 37.07
	296	PC11	COA	350	27.172	11.057	32.178	1.00 40.00
10	297	PC13	COA	350	28.667	11.476	32.189	1.00 40.00
	298	PC14	COA	350	26.632	11.101	30.745	1.00 40.00
	299	PC12	COA	350	26.933	9.588	32.579	1.00 40.00

***Mutants and Derivatives***

15 The invention further provides homologues, co-complexes and mutants of the *E. coli* FabH crystal structure of the invention.

The term "homologue" means a protein having at least 30% amino acid sequence identity with *E. coli* FabH or any of its functional domains.

20 The term "co-complex" means FabH or a mutant or homologue of FabH in covalent or non-covalent association with a chemical entity or compound.

The term "mutant" refers to a FabH polypeptide, i.e., a polypeptide displaying the biological activity of wild-type FabH activity, characterized by the replacement of at least one amino acid from the wild-type FabH sequence. Such a mutant may be prepared, for example, by expression of *E. coli* FabH cDNA previously altered in its 25 coding sequence by oligonucleotide-directed mutagenesis.

*E. coli* FabH mutants may also be generated by site-specific incorporation of unnatural amino acids into the FabH proteins using the general biosynthetic method of C. J. Noren et al, *Science*, 244:182-188 (1989). In this method, the codon encoding the amino acid of interest in wild-type FabH enzyme is replaced by a "blank" nonsense codon, TAG, 30 using oligonucleotide-directed mutagenesis. A suppressor tRNA directed against this codon is then chemically aminoacylated *in vitro* with the desired unnatural amino acid. The aminoacylated tRNA is then added to an *in vitro* translation system to yield a mutant FabH enzyme with the site-specific incorporated unnatural amino acid.

Selenomethionine may be incorporated into wild-type or mutant FabH by 35 expression of *E. coli* FabH -encoding cDNAs in auxotrophic or non- auxotrophic *E. coli* strains [W. A. Hendrickson et al, *EMBO J.*, 9(5):1665-1672 (1990)]. In this method, the wild-type or mutagenized FabH cDNA may be expressed in a host organism on a growth

medium depleted of either natural methionine but enriched in selenomethionine. The location(s) of the Se atom(s) can be determined by X-ray diffraction analysis at three or four different wavelengths. This information, in turn, is used to generate the phase information used to construct three-dimensional structure of the enzyme.

5      **II. Methods of Identifying Inhibitors of the Novel FabH Crystalline Structure**

Another aspect of this invention involves a method for identifying inhibitors of a FabH enzyme characterized by the crystal structure and novel active site described herein, and the inhibitors themselves. The novel *E. coli* FabH crystalline structure of the invention, or the structure of *E. coli* FabH homologue, permits the identification of inhibitors of FabH 10 activity. Such inhibitors may be competitive, binding to all or a portion of the active site of FabH; or non-competitive and bind to and inhibit FabH whether or not it is bound to another chemical entity.

One design approach is to probe the FabH crystal of the invention with molecules composed of a variety of different chemical entities to determine optimal sites for 15 interaction between candidate inhibitors and the enzyme. For example, high resolution X-ray diffraction data collected from crystals saturated with solvent allows the determination of where each type of solvent molecule sticks. Small molecules that bind tightly to those sites can then be designed and synthesized and tested for their FabH inhibitor activity [J. Travis, *Science*, **262**:1374 (1993)].

20      This invention also enables the development of compounds that can isomerize to short-lived reaction intermediates in the chemical reaction of a substrate or other compound that binds to or with FabH. Thus, the time-dependent analysis of structural changes in FabH during its interaction with other molecules is permitted. The reaction intermediates of FabH can also be deduced from the reaction product in co-complex with FabH. Such 25 information is useful to design improved analogues of known FabH inhibitors or to design novel classes of inhibitors based on the reaction intermediates of the enzyme and enzyme-inhibitor co-complex. This provides a novel route for designing FabH inhibitors with both high specificity and stability.

Another approach made possible by this invention, is to screen computationally 30 small molecule data bases for chemical entities or compounds that can bind in whole, or in part, to the FabH enzyme. In this screening, the quality of fit of such entities or compounds to the binding site may be judged either by shape complementarity or by estimated interaction energy [E. C. Meng et al, *J. Comp. Chem.*, **13**:505-524 (1992)].

Because FabH may crystallize in more than one crystal form, the structure coordinates of FabH, or portions thereof, as provided by this invention are particularly useful to solve the structure of those other crystal forms of FabH. They may also be used to solve the structure of FabH mutant co-complexes, or of the crystalline form of any other 5 protein with significant amino acid sequence homology to any functional domain of FabH.

One method that may be employed for this purpose is molecular replacement. In this method, the unknown crystal structure, whether it is another crystal form of FabH, a FabH mutant, a FabH co-complex, a FabH from a different bacterial species, or the crystal 10 of some other protein with significant amino acid sequence homology to any domain of FabH, may be determined using the FabH structure coordinates of this invention as provided in Figure 1 and Tables I - III. This method will provide an accurate structural form for the unknown crystal more quickly and efficiently than attempting to determine such information *ab initio*.

Thus, the FabH structure provided herein permits the screening of known molecules 15 and/or the designing of new molecules which bind to the structure, particularly at the active site or substrate binding site, via the use of computerized evaluation systems. For example, computer modeling systems are available in which the sequence of the FabH, and the FabH structure (i.e., the atomic coordinates, bond distances between atoms in the active site region, etc. as provided by Figures 1-2 and Tables I - III herein) may be input. Thus, a 20 machine readable medium may be encoded with data representing the coordinates of Figures 1-2 and Tables I - III. The computer then generates structural details of the site into which a test compound should bind, thereby enabling the determination of the complementary structural details of said test compound.

More particularly, the design of compounds that bind to or inhibit FabH according 25 to this invention generally involves consideration of two factors. First, the compound must be capable of physically and structurally associating with FabH. Non-covalent molecular interactions important in the association of FabH with its substrate include hydrogen bonding, van der Waals and hydrophobic interactions.

Second, the compound must be able to assume a conformation that allows it to 30 associate with FabH. Although certain portions of the compound will not directly participate in this association with FabH, those portions may still influence the overall conformation of the molecule. This, in turn, may have a significant impact on potency. Such conformational requirements include the overall three-dimensional structure and orientation of the chemical entity or compound in relation to all or a portion of the binding

site, e.g., active site or substrate binding sites of FabH, or the spacing between functional groups of a compound comprising several chemical entities that directly interact with FabH.

The potential inhibitory or binding effect of a chemical compound on FabH may be

analyzed prior to its actual synthesis and testing by the use of computer modelling

5 techniques. If the theoretical structure of the given compound suggests insufficient interaction and association between it and FabH, synthesis and testing of the compound is obviated. However, if computer modelling indicates a strong interaction, the molecule may then be synthesized and tested for its ability to bind to FabH and inhibit using a suitable assay. In this manner, synthesis of inoperative compounds may be avoided.

10 An inhibitory or other binding compound of FabH may be computationally evaluated and designed by means of a series of steps in which chemical entities or fragments are screened and selected for their ability to associate with the individual binding pockets or other areas of FabH.

15 One skilled in the art may use one of several methods to screen chemical entities or fragments for their ability to associate with FabH and more particularly with the individual binding pockets of the FabH active site or accessory binding sites. This process may begin by visual inspection of, for example, the active site on the computer screen based on the FabH coordinates in Figures 1-2 and Tables I - III. Selected fragments or chemical entities may then be positioned in a variety of orientations, or docked, within a binding pocket of  
20 FabH. Docking may be accomplished using software such as Quanta and Sybyl, followed by energy minimization and molecular dynamics with standard molecular mechanics force fields, such as CHARMM and AMBER.

Specialized computer programs may also assist in the process of selecting fragments or chemical entities. These include:

25 1. GRID [P. J. Goodford, "A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules", J. Med. Chem., 28:849-857 (1985)]. GRID is available from Oxford University, Oxford, UK.

2. MCSS [A. Miranker and M. Karplus, "Functionality Maps of Binding Sites: A Multiple Copy Simultaneous Search Method", Proteins: Structure, Function and Genetics, 11:29-34 (1991)]. MCSS is available from Molecular Simulations, Burlington, MA.

30 3. AUTODOCK [D. S. Goodsell and A. J. Olsen, "Automated Docking of Substrates to Proteins by Simulated Annealing", Proteins: Structure, Function, and

Genetics, 8:195-202 (1990)]. AUTODOCK is available from Scripps Research Institute, La Jolla, CA.

4. DOCK [I. D. Kuntz et al, "A Geometric Approach to Macromolecule-Ligand Interactions", J. Mol. Biol., 161:269-288 (1982)]. DOCK is available from 5 University of California, San Francisco, CA.

Once suitable chemical entities or fragments have been selected, they can be assembled into a single compound or inhibitor. Assembly may be proceed by visual inspection of the relationship of the fragments to each other on the three-dimensional image displayed on a computer screen in relation to the structure coordinates of FabH. This 10 would be followed by manual model building using software such as Quanta or Sybyl.

Useful programs to aid one of skill in the art in connecting the individual chemical entities or fragments include:

1. CAVEAT [P. A. Bartlett et al, "CAVEAT: A Program to Facilitate the Structure-Derived Design of Biologically Active Molecules", in Molecular Recognition in 15 Chemical and Biological Problems, Special Pub., Royal Chem. Soc. 78, pp. 182-196 (1989)]. CAVEAT is available from the University of California, Berkeley, CA.

2. 3D Database systems such as MACCS-3D (MDL Information Systems, San Leandro, CA). This area is reviewed in Y. C. Martin, "3D Database Searching in Drug 20 Design", J. Med. Chem., 35:2145-2154 (1992).

3. HOOK (available from Molecular Simulations, Burlington, MA).

Instead of proceeding to build a FabH inhibitor in a step-wise fashion one fragment or chemical entity at a time as described above, inhibitory or other FabH binding compounds may be designed as a whole or "*de novo*" using either an empty active site or optionally including some portion(s) of a known inhibitor(s). These methods include:

25 1. LUDI [H.-J. Bohm, "The Computer Program LUDI: A New Method for the De Novo Design of Enzyme Inhibitors", J. Comp. Aid. Molec. Design, 6:61-78 (1992)]. LUDI is available from Biosym Technologies, San Diego, CA.

2. LEGEND [Y. Nishibata and A. Itai, Tetrahedron, 47:8985 (1991)]. LEGEND is available from Molecular Simulations, Burlington, MA.

30 3. LeapFrog (available from Tripos Associates, St. Louis, MO).

Other molecular modelling techniques may also be employed in accordance with this invention. See, e.g., N. C. Cohen et al, "Molecular Modeling Software and Methods for Medicinal Chemistry", J. Med. Chem., 33:883-894 (1990). See also, M. A. Navia and M. A. Murcko, "The Use of Structural Information in Drug Design", Current Opinions in

Structural Biology, 2:202-210 (1992). For example, where the structures of test compounds are known, a model of the test compound may be superimposed over the model of the structure of the invention. Numerous methods and techniques are known in the art for performing this step, any of which may be used. See, e.g., P.S. Farmer, *Drug Design*, 5 Ariens, E.J., ed., Vol. 10, pp 119-143 (Academic Press, New York, 1980); U.S. Patent No. 5,331,573; U.S. Patent No. 5,500,807; C. Verlinde, *Curr. Biol.*, 2:577-587 (1994); and I. D. Kuntz, *Science*, 257:1078-1082 (1992). The model building techniques and computer evaluation systems described herein are not a limitation on the present invention.

Thus, using these computer evaluation systems, a large number of compounds may 10 be quickly and easily examined and expensive and lengthy testing avoided. Moreover, the need for actual synthesis of many compounds is effectively eliminated.

Once identified by the modelling techniques, the FabH inhibitor may be tested for bioactivity using standard techniques. For example, structure of the invention may be used in binding assays using conventional formats to screen inhibitors. One particularly suitable 15 assay format includes the enzyme-linked immunosorbent assay (ELISA). Other assay formats may be used; these assay formats are not a limitation on the present invention.

In another aspect, the FabH structure of the invention permit the design and identification of synthetic compounds and/or other molecules which are characterized by the conformation of FabH of the invention. Using known computer systems, the 20 coordinates of the FabH structure of the invention may be provided in machine readable form, the test compounds designed and/or screened and their conformations superimposed on the structure of the FabH of the invention. Subsequently, suitable candidates identified as above may be screened for the desired FabH inhibitory bioactivity, stability, and the like.

Once identified and screened for biological activity, these inhibitors may be used 25 therapeutically or prophylactically to block FabH activity, and thus, bacterial replication.

The following examples illustrate various aspects of this invention. These examples do not limit the scope of this invention which is defined by the appended claims.

Example 1 - The Expression of FabH from *Escherichia coli* in *Escherichia coli*.

30 The strategy for the expression of the FabH from *Escherichia coli*, using *Escherichia coli* as a host was based on the PCR amplification of the *fabH* gene and the introduction of suitable restriction sites that allowed the cloning of the *fabH*-containing DNA fragment in the pET29 expression vector. After the PCR amplification the insert of the resultant recombinant plasmid, (pET29c hereafter), was sequenced to verify the absence

of artifacts introduced by the *Taq* polymerase reaction. Expression was monitored by SDS-polyacrylamide gel analysis.

**A. Bacterial strains, Plasmids and Medium**

The *Escherichia coli* strains used were: MAXEfficiency DH10B

5 Competent Cells Genotype:  $F^- mcrA \Delta(mrr-hsdRMS-mcrBC) \phi80dlacZ\Delta M15 \Delta lacX74$  *deoR recA1 araD139*  $\Delta(ara, leu)7697 galU galK \lambda^+ rpsL nupG$ . *E. coli* cells were grown at 37°C in Luria Bertani broth (LB). These strains may all be obtained from commercial sources. BL21(DE3) competent cells for protein expression purchased by Novagen. The protocol used to make them electroporation-competent was the one provided by Invitrogen.

10 The plasmid used was pET29 [Novagen]. A detailed description of pET29 is provided in Figure 2. Briefly, pET29 is an expression vector of *E.coli* which is based on the T7 promoter-driven system and allows the selection of the recombinant clones by kanamycin resistance.

15 **LB Medium. Per liter:**

Bacto-tryptone	10 g
Bacto-yeast extract	5 g
NaCl	5 g

For plasmid propagation 0.1mg/ml of kanamycin was added to the medium.

**B. DNA manipulations**

20 Plasmid DNA was prepared by the rapid alkaline method (Sambrook et al, 1989). Transformations of *E. coli* cells were carried out according to the protocol provided with the DH10B or the electroporation method. The plasmids for sequencing were purified using QIAGEN mini-prep kit [QIAGEN]. DNA sequencing was carried out on supercoiled plasmid DNA by the dideoxy chain-termination method using the Thermo Sequenase cycle sequencing kit [ABI, BigDye, Applied BioSystems, USA]. Synthetic oligonucleotides [ordered in-house] were used as primers. Restriction enzymes and T4 DNA ligase were obtained from Gibco BRL (Life Technologies) and the experiments were carried out following the instructions provided by the suppliers.

25

The *fabH* gene from *E.coli* cloned in the pET29 vector was amplified by PCR using the primers:  
30 (5'-TATACATATGTATACGAAGATTATTGGT-3'; SEQ ID NO:2) and:

(5'-ATATGGATCCCTAGAAACGAACCAGCGCGG-3'; SEQ ID NO:3).

*NdeI* and *BamHI* restriction sites were incorporated at the 5' and 3' ends respectively of each primer to facilitate ligation of the amplified DNA into vectors.

5 Plasmid DNA (480 ng) was amplified in 100  $\mu$ l of PCR mixture containing 200  $\mu$ M deoxynucleotide triphosphates (dNTPs), 0.20 mM oligonucleotide primers, the manufacturer's buffer and 2.5 U of *pfu* (Stratagene). The following cycling parameters were used:

94°C 4 min

94°C 1 min, 55°C 40 sec, 72°C 1 min (30 cycles)

10 72°C 2 min

4°C

Polymerase chain reaction (PCR) was performed using the GeneAmp, PCR System 2400 [Perkin Elmer Cetus Co]. PCR-amplified DNA fragments were purified using Qiaquick PCR Purification kit for Rapid Purification of DNA Fragments [Qiagen].

15 **C. Cloning of the *fabH* gene of *E. coli* in the expression vector pET29 of *E. coli*.**

The cloning strategy is shown in Figure 2. PCR amplification of the *fabH* gene from *E. coli* using the primers AKK2 and AKK3 resulted in a DNA fragment of about 960 bp. This fragment was purified with Qiaquick PCR purification kit protocol (Qiagen) digested with *NdeI* and *BamHI*, purified, ligated overnight to the *NdeI* and *BamHI* sites of 20 already digested vector pET29 to obtain the recombinant plasmid pET29c. The ligation mix was used to transform *E. coli* DH10B competent cells. The construction of pET29c was initially confirmed by restriction analysis of the plasmid purified from the transformants. The amplification with *Taq* DNA polymerase made the sequencing of the *fabH* of pET29c an obligatory step to confirm that no changes were introduced due to the 25 low fidelity of this enzyme. Sequence analysis was accomplished by using T7 promoter and terminator primers. The sequencing of both strands showed that no artifacts had been introduced during the amplification process.

**D. Small-scale production of FabH from *E. coli* in *E. coli***

30 The plasmid pET29c and the negative control pET29 (vector without insert) were used to transform the *E. coli* BL21(DE3) host strain. Single clones of BL21(DE3): pET29c cells were grown overnight at 37°C in 2 ml of LB medium in the presence of 0.1 mg/ml kanamycin. The cells were then diluted 100-fold in 10 ml LB with kanamycin. When the cultures reached a value of 0.5 at OD<sub>600</sub> the *fabH* expression was induced by addition of isopropyl-thio-galactoside (IPTG) at 0.5 mM of final concentration.

After this induction 2 ml samples were taken at different times (1 and 2 hours). The cells were harvested in a microfuge for 3 min, the pellets were washed with 20 mM Tris-HCl pH 8, 1mM PMSF and resuspended in 100 ul of SDS-PAGE gel-loading buffer. The cells were broken by sonication (15 seconds). The samples were then boiled for 10 minutes and after 5 one spin, 10 ml fractions were analyzed by SDS-PAGE according to the methods of Laemmli [U. K. Laemmli, *Nature* 227, 680-685 (1970)]. The 4-12% polyacrylamide gels [NOVEX] were stained with Coomassie blue. As shown in Figure 3 good expression levels were detected from the early stages after induction with IPTG. The evidence was the presence of a prominent band (lanes 2, 4 and 6 in Figure 3) that was in good agreement 10 with the  $M_r$  predicted from the primary sequence. The FabH protein has a theoretical molecular weight of about 33,514 Da.

Example 2 - Large Scale Growth and Purification of FabH

**A. Large Scale Growth**

A 4 liter fermentation of *E.coli* BL21(DE3): pET29c was carried out in 15 Luria Bertani medium (LB), containing 100 ug/ml kanamycin. The 8x500 ml flasks were inoculated at 1% (v/v) from an overnight secondary seed culture in single strength LB medium, containing 100 mg/ml kanamycin. The flasks were incubated at 37°C, agitated at 250 rpm in a benchtop shaker. The OD at 600 nm was monitored, and at 0.5 absorbance 20 units, FabH expression was induced with the addition of isopropyl-thiogalactosidase to 0.5 mM and the cells harvested by centrifugation in a Sorval CSA rotor, 2 hours post induction. A total of 20 grams of cell paste was recovered.

LB Medium, per liter, contains the following components. The medium was supplied by the in-house laboratory.

Single strength

25 Bacto Tryptone 10 g  
Bacto Yeast Extract 5 g  
Sodium Chloride 5 g

**B. Purification**

**1) Lysis**

30 12.5 g of cells of *E. coli* overexpressing *E. coli* FabH obtained as described above, were resuspended in 140 ml of 20 mM Tris pH 7.9, 50 mM NaCl, 1mM EDTA, 1mM DTT, 10% glycerol, 1 mM PMSF (buffer A). Lysozyme ( Sigma Chemicals: hen egg) was added to a final concentration of 1 mg/ml. Cells were incubated at 37°C for 20min. The cells were then lysed by sonication in an ice water bath (4x30sec). DNase

(Sigma; bovine pancreas type 1) was added along with MgCl<sub>2</sub> and held at 37°C for 5 minutes. The solution was centrifuged in a Beckman JA-HS centrifuge at 14,000 g for 60 minutes using a Beckman JA-14 rotor.

2) Anion exchange

5 All chromatography was performed on a Pharmacia chromatography system, fitted with a UV detector (Pharmacia, Monitor UV-1). UV (at 280 nm) was monitored during all operations. All operations were performed at 4°C.

10 The supernatant from 1) was loaded onto a Q-Sepharose high performance (Pharmacia) column of 50 ml packed into a Pharmacia XK-26 column. The column was equilibrated with buffer A prior to loading. The column was then washed with buffer A (250 ml) at 4 ml/min, and eluted with a linear gradient of buffer A to 1M NaCl in buffer A over 80 minutes at 4 ml/min. The eluate was fractionated into 8 ml fractions using a Pharmacia FRAC 200.

15 The eluted fractions were assayed for FabH activity by measurement of incorporation of [<sup>14</sup>C]Acetyl-CoA to Malonyl-ACP and , and for protein by the Bradford method. Active fractions were analyzed by reducing SDS-PAGE (NOVEX, NuPAGE Bis-Tris 4-12 % gradient gel). Active fractions pooled together and dialyzed against Buffer A.

20 3) Anion Exchange chromatography

25 The dialyzed material was loaded onto a MonoQ column equilibrated with buffer A (Pharmacia, 5/5). The column was washed with 20 ml of the equilibration buffer until 280 nm absorbance returned to base line and then eluted with a linear gradient of equilibration buffer to buffer A over 90 minutes at 0.5 ml/min. Fractions were pooled together, collected, assayed for FabH activity.

30 4) Hydroxyapatite/ buffer exchange

35 Eluted fractions are collected (1 ml fraction) and assayed for FabH activity and protein. Active fractions are pooled and the volume was doubled with Buffer B [20 mM Tris-HCl pH 7.4, 50 mM NaCl, 1 mM DTT and 10% glycerol] to reduce the salt concentration in half. The active eluate was loaded in a hydroxyapatite column and eluted with 0.5 M Potassium Phosphate pH 7.4. The active enzyme was buffer exchanged with 20 mM Tris pH 7.4, 50 mM NaCl 2 mM DTT. This product was greater than 97% purity by SDS PAGE and the activity showed an overall process yield of 60 % from 1). N-terminal amino acid analysis confirmed identity.

Example 3 - Fermentation, Purification and Characterization of seleno-methionine derivative of *Escherichia coli* FabH

**A. Fermentation**

To obtain soluble selmet-FabH for purification and crystallization studies, *E. coli* strain 5 BL21 (DE3) was transformed with pET29c FabH. 50 ul of the seed culture expressing FabH gene product was inoculated into 100 ml of Luria broth, containing kanamycin (50 ug/ml) and glucose (0.6%). On reaching target density of 2 OD, the cells from the seed culture were isolated by centrifugation, resuspended in 100 ml of M9 minimal medium containing 1 mM CaCl<sub>2</sub>, 1 mM MgSO<sub>4</sub>, kanamycin ( 50 ug/ml) and glucose (0.6% w/v). 10 The resuspended pellets were then added to 900 ml of the same medium and the cells were grown at 37 C to mid-log phase, A<sub>600</sub> of 1.5, at which point lysine, phenylalanine, threonine at 100 mg/l each, and selenomethionine, isoleucine, leucine, and valine at 50 mg/l were added. The culture was shaken for 15 minutes, and then induced with 0.5mM isopropyl  $\beta$ -D-thiogalactopyranoside (IPTG). The culture was grown for 13 hours, and 15 harvested by centrifugation (speed). 5 ml aliquots were taken prior to and during induction to monitor the expression of selenomethionine FabH. 12g of cell paste (wet wt) was recovered from 5L. In addition, to compare the expression of selenomethionine derivative to that of wt FabH, a one l culture was prepared under identical conditions except that the cells were grown in LB media.

20 **B. Purification**

All lysis and purification steps were carried out using degassed buffers in a cold room or on ice. 4.5 g of *E. coli* cells over expressing Fab H were resuspended in 50 ml of 20 mM Tris, 50 mM sodium chloride, 10% glycerol, 0.2 mM PMSF, 2 mM DTT, pH 7.9 (buffer A). Cells were lysed twice at 10,000 psi using Microfluidizer (Microfluidics Corporation, MA). 25 Cell debris was removed by centrifugation (Sorvall RC-5B) at 35,000 g for 30 min. The supernatant was applied to a 2.6 x 4 cm Source Q column (Pharmacia) equilibrated in buffer A. The column was washed with 10 column volumes of buffer A, and eluted with a 10 column volume gradient of 0 to 1.0 M NaCl in buffer A. Eluted fractions were analyzed by 10% SDS-PAGE under reducing conditions. Fab H eluted at 0.2 - 0.25 M NaCl. Fab H 30 containing fractions were pooled and applied to a 2.6 x 6 cm Hydroxyapatite column (Bio-Rad, Type I, 40u) equilibrated in buffer A. The column was eluted with a 30 column volume linear gradient of buffer A to 400 mM potassium phosphate, 10% glycerol, 2 mM DTT. Fab H, which eluted at 80-180 mM potassium phosphate, was diluted 1:2 with 50 mM Tris, 200 mM NaCl, 10% glycerol, 2 mM DTT, pH 7.5 (buffer B) and applied to a 1.6 X 7.5

cm Blue Sepharose column (Pharmacia) equilibrated in buffer B. The column was eluted with buffer B containing 1 M NaCl. Blue Sepharose eluted Fab H fractions were next applied to a 2.6 x 60 cm Superdex 200 size exclusion column (Pharmacia) equilibrated in 20 mM Tris, 50 mM NaCl, 2 mM DTT, pH 7.4. A total of 23 mg of Fab H was recovered 5 which was concentrated to 13 mg/ml using Amicon 3 filtration device.

### C. Characterization

#### i). Mass Spectroscopy

10 Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) data were obtained on a PerSeptive Biosystems Voyager RP laser desorption time-of-flight mass spectrometer. Protein samples were prepared for analysis by diluting analyte 1:5 with 3,5-Dimethoxy-4-hydroxy-cinnamic acid (10mg/ml in 2:1 0.1% trifluoroacetic acid/15 acetonitrile) for a final concentration of 1-10 pmol/ul. Bovine Beta lactoglobulin A (Sigma) was included as an internal calibrant ( $MH^+$  18364 Da). Desorption/ionization was accomplished using photon irradiation from a 337-nm pulsed nitrogen laser and 30-keV accelerating energy. Spectra were averaged over ca. 100 laser scans.

15 The predicted molecular mass for native FabH is 33516 Da. MALDI-MS analysis of the selenomethionyl incorporated FabH protein construct provided a mass of 33,889 Da. This is in close agreement with the predicted +375Da shift in mass expected for the sulfur to 20 selenium side-chain substitution of eight methionine residues within the protein (33,891 Da theoretical).

#### ii). N-terminal sequence analysis

25 Sequence analysis was performed on a Hewlett-Packard G1000A N-terminal Protein Sequencer with on-line PTH identification using an HP1100 HPLC. Samples were applied directly to biphasic sequencing cartridges and standard 3.1 sequencing method cycles were used.

30 N-terminal sequencing results showed negligible native methionine in the first residue. Instead, a unique PTH (phenylthiohydantoin) derivative was observed which eluted 1.6 minutes later than PTH-methionine, and did not coelute with any natural PTH-amino acid derivatives. The increase in hydrophobicity is consistent with the direct detection of the PTH-selenomethionyl amino acid derivative.

### D. Measurement of $\beta$ -ketoacyl-ACP synthase III activity.

The enzyme catalyses the condensation of acetyl-CoA with malonyl-ACP to form acetoacetyl-ACP. The reaction can be described by three distinct steps: (a) the acyl group

of acetyl-CoA is transferred to the active site cysteine resulting in a acyl-enzyme thioester; (b) carbanion formation by the decarboxylation of malonyl-ACP; and (c) carbon-carbon bond formation by nucleophilic attack of the carbanion onto the carbonyl carbon atom of the acyl-enzyme thioester to yield the acetoacetyl-ACP product.

5 This reaction can be assayed in order to characterize the enzyme or identify specific inhibitors of its activity in two ways:

(1) Radiolabeled acetoacetyl-ACP formation can be specifically determined using [3H]-acetyl-CoA and malonyl-ACP. The [3H]-acetyl-CoA substrate is soluble in 10%TCA while the resulting [3H]-acetoacetyl-ACP is not. A reaction mixture containing 100mM 10 sodium phosphate buffer pH7.0, 1mM DTT, 34uM acetyl-CoA, 0.15uM [3H]-acetyl-CoA (specific activity 25Ci/mmol), and 7uM malonyl-ACP is prepared and transferred to a microtiter plate with or without inhibitors already added. The enzyme is added last to start the reaction and the plate is incubated at 37 degrees C. Ten percent TCA is added to stop the reaction, and then 50ug of BSA as a carrier. Stopped reactions are filtered and washed 15 2 times with 10% TCA on Wallac GF/A filtermats using a TomTec harvester. The filtermats are dried at 60 degrees C and the radioactivity quantified using Wallac Betaplate scintillation cocktail and a Wallac Microbeta 1450 liquid scintillation counter. IC50s are generated using Grafit 4.0 software and solved using the equation  $v = V_{max}/(1 + I/IC50)$ .

20 (2) FabG coupling can also be used to measure FabH production of acetoacetyl-ACP by following NADPH consumption at 340nm. FabG ( $\beta$ -ketoacyl-ACP reductase) will specifically reduce the C3 carbonyl of acetoacetyl-ACP to form  $\beta$ -hydroxybutyryl-ACP. This reduction requires the conversion of NADPH to form NADP+ which can be monitored by following the optical density at wavelength 340nm.

25 (3) FabD coupling is an available assay option in the absence of purified malonyl-ACP. FabD (Malonyl-CoA:ACP transacylase) is responsible for malonic acid transfer from malonyl-CoA to ACP to form malonyl-ACP. This activity can be exploited by applying the techniques described in (1) above together with *de novo* malonyl-ACP from the FabD reaction.

30

**E. Ligand binding to FabH.**

It is also possible to define ligand interactions with FabH in experiments that are not dependent upon enzyme catalyzed turnover of substrates. This type of experiment can be done in a number of ways:

5 (1) Effects of ligand binding upon enzyme intrinsic fluorescence (e.g. of tryptophan). Binding of either natural ligands or inhibitors may result in enzyme conformational changes which alter enzyme fluorescence. Using stopped-flow fluorescence equipment, this can be used to define the microscopic rate constants that describe binding. Alternatively, steady-state fluorescence titration methods can yield the overall dissociation constant for binding in the same way that these are accessed through enzyme inhibition  
10 experiments.

15 (2) Spectral effects of ligands. Where the ligands themselves are either fluorescent or possess chromophores that overlap with enzyme tryptophan fluorescence, binding can be detected either via changes in the ligand fluorescence properties (e.g. intensity, lifetime or polarization) or fluorescence resonance energy transfer with enzyme tryptophans. The ligands could either be inhibitors or variants of the natural ligands.

20 (3) Thermal analysis of the enzyme:ligand complex. Using calorimetric techniques (e.g. Isothermal Calorimetry, Differential Scanning Calorimetry) it is possible to detect thermal changes, or shifts in the stability of FabH which reports and therefore allows the characterization of ligand binding.

**Example 3 - Crystallization of *E. coli* wild-type and selenomethionine mutant of FabH**

**A. Crystallization**

25 All crystals were grown at room temperature using the sitting-drop vapor diffusion method. The drop solution was always a 1:1 mixture of the protein sample and the well solutions. For the crystal form 1 of the wild-type protein, the well solution contained 0.1 M HEPES buffer at pH 7.5 and 20% PEG8000. For the crystal form 2 of the selenomethionine mutant protein in complex with acetyl-CoA, the well solution contained 0.05 M Bis-Tris propane buffer at pH 7.0, 0.1 M MgCl<sub>2</sub> and 14% PEG6000. Crystals grew overnight and  
30 are approximately 0.1 to 0.2 mm in sizes.

**B. X-ray Diffraction Characterization**

All crystals were frozen in liquid nitrogen streams before their characterization using synchrotron X-ray radiation. Diffraction data for the apo form 1 crystal was collected to 2.0 Å resolution. The data is 97.1 % complete and 6 fold redundant with a merging R-

factor of 7.7 %. The crystal belongs to the orthorhombic spacegroup P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, with cell dimensions a = 63.1, b = 65.1 and c = 166.5 Å. For the Se-Met protein in complex with acetyl-CoA, data were collected at three different wavelengths: 0.9789, 0.9785 and 0.9414 Å. The three data set were of nearly identical quality, with about 80% completion, 6-fold 5 redundancy, 8.5 % merging R-factor, and 1.9 Å resolution. The form 2 crystal belongs to the tetragonal spacegroup P4<sub>1</sub>2<sub>1</sub>2, with a = b = 72.4 and c = 102.8 Å.

#### C. Structure Solution

10 The crystal structure of the Se-Met *E. coli* FabH mutant in complex with acetyl-CoA was solved to 1.9 Å resolution using the MAD phasing technique with the data sets collected at three different wavelengths and the program SOLVE (Terwilliger & Berendzen, 1999, *Acta Cryst. D55*, 849-861). All eight Se-Met were located by SOLVE. The overall MAD phasing figure of merit was 0.6 to 1.9 Å resolution, and the overall Z score was as high as 148. The resulting electron density map was of very high quality. The 15 structure of the apo enzyme (crystal form 1) was solved with the molecular replacement method using the acetyl-CoA complex structure as the search model. This crystal form had a FabH dimer in the asymmetric unit, and the R-factor of the solution was only 33%. Two-fold averaged map was then calculated and used for model building.

#### D. Model Building and Refinement

20 The electron density for the acetyl-CoA complex was very clear and a structure model for the whole FabH protein, the bound acetyl group and CoA, as well as 98 solvent molecules were built in the first round. Standard structural refinement protocols and manual model building led to the current model, which has an R-factor of 27 % to 1.9 Å resolution. The model for the apo FabH structure was also built readily, and refined to an 25 R-factor of 18.9 % (R<sub>free</sub> of 24.4%) to 2.0 Å resolution. Both models have excellent geometry and do not have any outliers in the Ramanchandran plot, indicating high quality of the atomic coordinates, which contain an estimated error of less than 0.3 Å.

30 This invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims. The disclosures of the patents, patent applications and publications cited herein are incorporated by reference in their entireties.